UNITED STATES DEPARTMENT OF AGRICULTURE
SMALL BUSINESS INNOVATION RESEARCH
SOLICITATION NO. USDA / 97-1
PHASE I AND PHASE II
PROPOSAL COVER SHEET

9.1
OMB Approved 0524-0025
Expires 5/98

Proposal No.
Date Received

SUBMITTED BY	Firm: Aquaculture Systems Technologies, LLC
	Mailing Address: P.O. Box 15827, New Orleans, LA 70175

Project Title: "High frequency Wash airlift Bioclarifier Using Modified Floating Media for Recirc Systems"

Topic No. and Area (check appropriate box; see Section 8.0)

- ☐ 8.1 Forests and Related Resources
- ☐ 8.2 Plant Production and Protection
- ☐ 8.3 Animal Production and Protection
- ☐ 8.4 Air, Water, and Soils
- ☐ 8.5 Food Science and Nutrition
- ☐ 8.6 Rural and Community Development
- ☒ 8.7 Aquaculture
- ☐ 8.8 Industrial Applications
- ☐ 8.9 Marketing and Trade

Amount Requested ($) 197,904.32 · Proposed Duration (Mos.) 24 · Congressional District No. 2nd	YES	NO
1. The above concern certifies that it is a small-business firm and meets the definition as stated in this solicitation (See Subsection 2.2).	X	
2. The above concern certifies that it qualifies as a socially and economically disadvantaged small business as defined in this solicitation (See Subsection 2.4). (For statistical purposes only).		X
3. The above concern certifies that it qualifies as a women-owned small business as defined in this solicitation (See Subsection 2.5). (For statistical purposes only).	X	
4. The above concern certifies that the Principal Investigator's primary employment will be with proposing firm at the time of any resulting award and during the conduct of the proposed research (See Subsection 2.2(c)).	X	
5. The above concern certifies a minimum of two-thirds of the research (phase I) or one-half the research (phase II) will be performed by this firm.	X	
6. Will you permit the Government to disclose the title and technical abstract page of your proposed project, plus the name, address, and telephone number of the corporate official of your firm, if your proposal does not result in an award, to entities that may be interested in contacting you for future information?	X	
7. Do you plan to send, or have you sent, this proposal or a similar one to any other Federal agency? If yes, give acronym(s); e.g., DOE, NIH, NSF, etc.		X
8. Is the organization delinquent on any Federal Debt? (See Subsection 5-14(G)). (If yes, attach explanatory information).		X
9. Will the work in this proposal involve recombinant DNA, living vertebrate animals, or human subjects?. (If yes, complete Form CSREES-662).	X	

By signing and submitting this proposal, the prospective grantee is providing the required certifications set forth in 7 CFR Part 3017, as amended, regarding Debarment and Suspension and Drug-Free Workplace; and 7 CFR Part 3018 regarding Lobbying. (Please read the Certifications and Instructions included in this solicitation before signing this form.) In addition, the prospective grantee certifies that the information contained herein is true and complete to the best of its knowledge and accepts as to any grant award, the obligation to comply with the terms and conditions of the Cooperative State Research, Education, and Extension Service in effect at the time of the award. *Submission of the Social Security Number is voluntary and will not affect the organization's eligibility for an award. However, it is an integral part of the CSREES information system and will assist in the processing of the proposal.

PRINCIPAL INVESTIGATOR	AUTHORIZED ORGANIZATIONAL OFFICIAL
Name and Social Security Number*: Douglas G. Drennan	Name: Douglas G. Drennan
Title: Managing Member	Title: Managing Member
Address: P.O. Box 15827, New Orleans, LA 70175	Address: P.O. Box 15827, New Orleans, LA 70175
Telephone No.: (504) 837-5575 · Fax No.: (504) 837-5585	Telephone No.: (504) 837-5575 · Fax No.: (504) 837-5585
Signature: · Date: 2/12/97	Signature: · Date: 2/12/97

PROPRIETARY NOTICE (IF APPLICABLE, SEE SUBSECTION 5.4)

The following pages (specify) contain proprietary information which (name of proposing organization) requests not be released to persons outside the Government, except for purposes of evaluation.

Form CSREES-667 (4/95)

The following (Task 1, page 15, 16, 17, 18, 19, and Figures 7 (page 18)) contain proprietary information which Aquaculture Systems Technologies, LLC requests not be released to persons outside the Government, except for purposes of evaluation.

U.S. DEPARTMENT OF AGRICULTURE
SMALL BUSINESS INNOVATION RESEARCH
PHASE I AND PHASE II
PROJECT SUMMARY*

9.2
OMB Approved 0524-0025
Expires 5/98

FOR USDA USE ONLY

Program Office	Solicitation No.	Proposal No.	Topic No.

TO BE COMPLETED BY PROPOSER

Name and Address of Firm	Name and Title of Principal Investigator(s)
Aquaculture Systems Technologies, LLC P.O. Box 15827 New Orleans, LA 70175	Douglas Drennan Managing Member

Title of Project (80-character maximum) "High Frequency Wash Airlift Bioclarifier Using Modified Floating Media for Recirc Systems"

Technical Abstract (200-word limit)

Recent research findings during Phase I of this project have led the research team to conclude that optimum nitrification performance in floating bead filters will only occur in filters subjected to high frequency washing, in which the adverse impacts of solids accumulation are virtually eliminated. The solids not only break down to produce ammonia (Matsuda et al., 1990), but also encourage rapid heterotrophic bacteria growth that competes with the nitrifiers for space, potentially limiting nutrients, and oxygen (Bovendeur et al, 1990). The beads will have to be re-formed to store increased volumes of nitrifying bacteria at loadings in excess of 2 Lbs/day-ft^3 without a reduction in hydraulic conductivity. The beads will also have to provide for abrasion protection in aggressively washed formats, but, excessive protection should be avoided. In summary, this project proposes to continue the development of a highly specialized bioclarifier which will reduce the water reconditioning costs associated with the holding, breeding, or production of aquatic animals in recirculating aquaculture systems.

Anticipated Results/Potential Commercial Applications of Research (100-word limit)

AST believes that the future for recirculating technologies is bright. Many factors, including issues as diverse as bird predation, diminishing water supply, environmental regulations, cost of coastal lands, concerns about exotic introductions, and quality issues such as off-flavor are driving the aquaculture industry towards increased production, at least in part based on recirculating formats. The key to success will be the availability of reliable and cost effective production technologies. Phase II will continue to address the scientific issues, apply findings to development of new technologies, which we believe will lead directly to the implementation of a new generation of airlift bead filters or "bioclarifiers" utilizing modified floating media.

Keywords to Identify Technology/Research Thrust/Commercial Application (8-word maximum)

High Frequency Wash
Airlift
Bioclarifier
Modified Floating Media

*The Project Summary must be suitable for publication by USDA in the event of an award. Do not include proprietary information on this page.

Form CSREES-668 (7/87)

(C) TECHNICAL CONTENT

(1) Identification and Significance of the Problem or Opportunity

The rapidly expanding aquaculture industry must cope with a variety of socioeconomic issues which can dramatically affect its progress. These include such problems as increased competition for water rights, price depression caused by foreign imports, and predation by protected migratory birds. As the aquaculture industry grows, finding cost-effective ways to reduce pollution and improve waste management becomes an imperative for scientists and engineers working in the environmental arena. It is estimated that 80% of the feed supplied to an aquaculture system is eventually discharged to the environment through fish excretion (Hopkins and Manci, 1989), including a total phosphorus load ranging from 0.012 to 0.058 Kg/Kg fish/ year, and total nitrogen ranging from 0.045 to 0.077 Kg/Kg fish/year. In 1985, the world's aquacultural production was approximately 10.6 million metric tons (Nash, 1988). In the United States, aquaculture production increased more than 20% annually from 1980 to 1988 (Dicks and Harvey, 1988), and reached approximately 390 million kilograms in 1990 (Joint Subcommittee on Aquaculture, 1993). With this amount of aquacultural production, the environmental impact associated with its waste component is significant--and growing. Production systems based on flow-through technologies and extensive pond systems are particularly vulnerable to the rising public concern over conservation issues and the environment. On balance, current popular support for the principle of sustainability in the production of renewable resources argues for expanding the use and development of recirculating systems technology, which dramatically reduces the aquacultural producer's impact on public waters.

Current uses of recirculating systems are limited, in large part, to the production of high-value products for niche markets, but the picture is changing rapidly. In the near future, we expect that recirculating systems will be widely employed in specialized facilities that complement extensive (pond) growout operations in such applications as producing disease free fingerlings, overwintering warmwater broodstock, and purging off-flavors. Recirculating technologies also afford an alternative production strategy for operators of flow-through systems who must cope with limited or diminishing water supplies and stringent water quality discharge standards. Several large, recirculating production facilities are already attempting to compete directly with foreign imports and extensive domestic operations in the production of low cost foodfish such as the warmwater tilapia, although their long term economic viability has yet to be clearly demonstrated.

Given recent advances in technology (Timmons and Losordo 1994; Wheaton 1985), the main obstacles to widespread adoption of recirculating aquaculture technology seem to be related to economics (Losordo 1991; O'Rourke 1991; Losordo et al 1992). Systems that provide internal measures for dealing with the waste burden have had difficulty competing with technologies which do not, especially when the latter could benefit from natural subsidies represented by cheap or free water and waste disposal. In most parts of the country, these subsidies are either disappearing or under attack as water supplies diminish, development proceeds, and water uses become more and more subject to regulation. Generally speaking, the inclusion of a water reconditioning system to deal with the burden of fish wastes is what distinguishes the recirculating system from the traditional pond and flow-through technologies, and it is the additional capital and operating costs associated with this feature that must be addressed through technological refinements and re-engineering.

The economic barriers associated with recirculating systems seem to be yielding as experience in commercial settings accumulates. Aquaculture Systems Technologies, LLC (AST) has successfully advanced floating bead filters as bioclarifiers, which can accomplish both solids capture and nitrification economically and efficiently (Malone and Coffin 1991; Wimberly 1990; Cooley 1979). In the last 12 months, more than two hundred floating bead filters have been installed nationwide, indicating a growing acceptance of bead filter technology by the aquaculture community. The results of this work, with appropriate modifications, will contribute to a range of recirculating system applications including growout, broodstock maintenance, and fingerling production facilities for shrimp, tilapia, hybrid striped bass, red drum, ornamental fish and other economically valuable species.

Low head centrifugal pumps are most often used in aquaculture applications. These pumps are designed to deliver large volumes of water at moderately low lifts. The lift of a pump is a measure of the back pressure at the pump outlet during operation. It is expressed as head, i.e., the height of a water column (feet) that would create an equivalent back pressure, or directly in units of pressure (psi) at the pump outlet. Ideally, recirculating systems should be designed with low back pressure on their pumps to minimize energy requirements. Centrifugal pumps capable of delivering flows at pressures of 20-30 feet of water (8 to 13 psi) are most frequently selected for large scale recirculating applications.

Another approach to water circulation is the airlift pump. These simple pumps move water by exploiting the density difference induced when air is injected into a submerged vertical pipe. Practical only at low lift heights (0-5 feet), airlift pumps are cost effective. Airlift pumps can move large amounts of water, particularly at low lifts (0-2 ft). Their principal advantage stems from the fact that they also contribute to the aeration and degasification capacity of the system. Additionally, airlift pumps are simple to maintain since they contain no metal or moving parts, an important consideration in a wet environment. Because of their limited lift, airlift pumps are normally employed as part of an integrated design where all of the components are optimized to minimize head loss.

In summary, this project proposes to continue the development of a highly specialized bioclarifier which will reduce the water reconditioning costs associated with the holding, breeding, or production of aquatic animals in recirculating aquaculture systems. The potential implications of this technology are very far-reaching. The system will have an immediate impact on production costs associated with several high-value aquaculture industries including ornamental fish production, high density growout of tilapia, and, the purging and marketing of crawfish. In the near future, benefits of this technology may accrue to operators of extensive growout systems for catfish, shrimp, and marine finfish through use of recirculating systems to produce fingerlings, head start juveniles, and maintain broodstock.

(2) Background and Rationale

The term "recirculating" applies to many high density aquaculture systems which wholly or partially reuse water. The sophistication of the treatment block is largely controlled by the degree of water reuse. The term "turnover rate" is the most widely accepted measure of water reuse and is defined as the length of time required for the cumulative source water introductions to match the system's total water volume. The balance between costs and benefits will most frequently dictate the turnover rate associated with recirculating systems. As the water reuse intensifies with a declining water replacement rate, the complexity of the treatment train increases. The cost of treatment also rises, neutralizing the benefits. Culture systems found in the United States range in complexity from simple flow-through raceways--where only aeration is provided--to virtually no-discharge marine aquaria where even subtle shifts in trace elements are monitored. Most recirculating systems, however, address only the five major treatment processes: solids capture, biofiltration, aeration, degasification, and maintenance of critical ions while avoiding the long- term issues by allowing a slow water release at a rate of two to 10 percent daily (turnover rates of 50 to 10 days).

Bead filters are classified as "Expandable Granular Biofilters" (EGB's), which means that they are designed to function as physical filtration devices (or clarifiers), for removing solids, while simultaneously facilitating the growth of desirable bacteria which remove dissolved wastes from the water through the biofiltration process. These units are most appropriately described as "bioclarifiers". Their ability to serve as clarifiers and biofilters simultaneously simplifies system configurations (Malone and Delos Reyes, 1997) while reducing both capital and operating costs in comparison with many alternatives.

Most floating bead filters manufactured today consist of a confined bed of small, spherical, 2-3 mm diameter buoyant plastic (low density polyethylene) beads restrained by an overlying screen. Filter shapes vary widely. The filters are operated in the filtration mode most of the time. As the recirculating waters pass through the bed, suspended solids are captured and the biofiltration processes act on dissolved nutrients. Periodically, cleaning of the bead bed is accomplished by mechanical, hydraulic, or pneumatic means. The objective of the backwashing step is to release solids and excessive biofloc from the beads, thus restoring hydraulic conductivity. Sludge is removed with or without the benefit of settling, allowing initiation of another bioclarification cycle.

Bead filters capture solids through four identifiable mechanisms including straining, settling, interception, and adsorption. Bead filters perform well in the control of suspended solids across a broad spectrum of sizes with nearly 50 percent of fines in the 1-10 micron range being removed in a single pass (Ahmed, 1996). Although bead filters are inherently excellent clarifiers, their market demand is largely based on their ability to function as bioclarifiers. Thus, they can address two of the major process requirements as a single unit.

In the biofiltration mode, bead filters are classified as fixed film reactors. Each bead (Figure 1) becomes coated with a thin film of bacteria that extracts nourishment from the wastewater as it passes through the bed. There are two general classifications of bacteria, heterotrophic and nitrifying, that are of particular interest. The two bacteria co-exist in the filter, and understanding their impact on each other as well as on the filter is critical.

The classification of heterotrophic bacteria encompasses a great number of genera/species which share the common characteristics of extracting their nourishment from the breakdown (decay) of organic matter. Biochemical oxygen demand (BOD) is largely an indirect measure of the biodegradable organic material in water. Heterotrophic bacteria reduce BOD levels and consume oxygen in the process. About 60 percent of the organic matter consumed is converted to bacterial biomass, whereas the balance (40 percent) is converted to carbon dioxide, water, or ammonia. Heterotrophic bacteria grow very fast and are capable of doubling their populations every ten to fifteen minutes. If the BOD in the water being treated is very

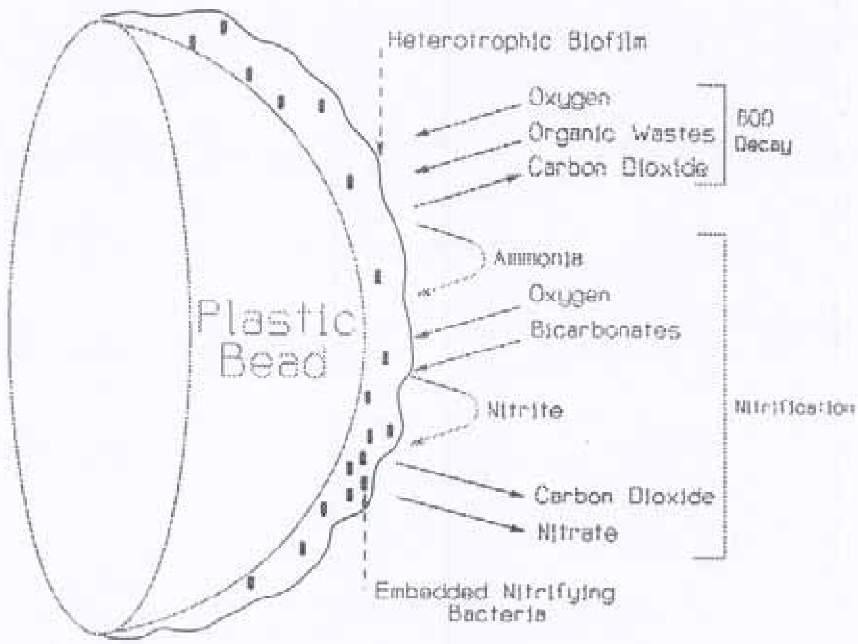

Figure 1. The heterotrophic bacteria form a thin biofilm layer on each bead. The nitrifying bacteria compete with the heterotrophic bacteria for space.

high (> 20 mg -O_2/l), the heterotrophs will quickly dominate the bead bed, overtaking the slower- growing nitrifying bacteria and consuming tremendous amounts of oxygen.

The second--yet most important--group of filter bacteria are the nitrifiers. These bacteria are specialists, extracting energy for growth from the chemical conversion of ammonia to nitrite and from nitrite to nitrate. Nitrate is a stable end product which, although a valuable nutrient for plants, displays little of the toxic impacts of ammonia and nitrite. Composed principally of two genera (Nitrosomonas and Nitrobacter), nitrifying bacteria are very slow growing and sensitive to a wide variety of water quality factors. It is not surprising that most bead filters used for biofiltration are managed to optimize conditions for nitrification.

The actual nitrification performance can be estimated by calculating the volumetric nitrification capacity (VNR, in units of grams TAN converted/cubic foot of beads per day) determined by measuring the influent TAN concentration (TAN_I in mg-N/l), the effluent TAN concentration (TAN_E in mg-N/l) and the flowrate through the filter (Q in gallons per minute) and entering them into Equation 1:

$$VNR = \frac{5,450\ (TAN_I - TAN_E)\ Q}{(V)} \qquad (1)$$

where V is the volume of beads in the filter (in ft^3), and 5,450 represents a conversion factor correcting for differences in units of the various terms. The peak sustainable nitrification rate that a filter can deliver under typical conditions for growout (TAN <1.0 mg/l) can be defined as volumetric nitrification capacity, or VNC. The VNC for the bead filters currently produced by AST is about 10 gm/ft^3-day. Since the sizing of bead filters is controlled by their VNC, the VNC effectively determines the suitability of a given filter for a particular application. Thus, the focus of this proposed effort is to refine a floating bead filter format (media characteristics and backwashing approach) that will maximize VNC.

The nitrification rate (VNR) of a filter can be generally described by a governing equation (Golz et al, 1996; Malone et al, 1993) which reflects the impact of exchange with the fish rearing tank, the kinetics of nitrification, and TAN production from solids decay:

$$d(AV_b)/dt = Q_r(A_T - A) - K_{max}\,X_n\,[A/(K_b + A)][DO/K_{DO} + DO] + K_{SN}\,S \qquad (2)$$

where,

V_b=the volume of water in the bead bed.
A=the TAN concentration in the bead bed (under a simplifying completely mixed assumption (mg-N/l).
Q_r=the exchange rate between the bioclarifier and holding tank.
A_T=the TAN concentration in the rearing tank (mg-N/l).
K_{max}=the maximum uptake rate (maximum growth rate/yield) (1/day).
X_n=the biomass of nitrifying bacteria (gms)
K_B=The apparent half saturation constant for TAN (mg-N/l)
DO=Dissolved oxygen concentration in the bead bed (mg/l)
K_{DO}=The apparent half saturation constant of oxygen (mg/l)
K_{sn}=A modified first organic nitrogen decay constant which compensates for the nitrogen fraction in S (1/days).
S=the mass of organic solids and biomass accumulated in the bead bed.

Most recirculating systems are operated under a substrate regime (TAN concentration) that is dictated by the finfish species being reared. Under these conditions, the net nitrification performance of floating bead filters is influenced by the amount of nitrifying biomass held in the filter (X_n), the flowrate delivered, and the dissolved oxygen levels available to the nitrifying bacteria in the biofilm. A significant offset can occur due to ammonification of accumulated solids in the biofilter.

The objective of a biofilm management program is to maximize the relative level of nitrifying bacteria found within a biofilm that is dominated by heterotrophic bacteria. The issue of heterotrophic bacterial competition is made complex by the fact that the nitrifying bacteria reproduce very slowly (particularly Nitrobacter). As organic loadings increase, the mass of heterotrophic bacteria increases, clogging the filter and forcing a washing sequence (Manthe et al., 1988). The washing sequence removes the bulk of the heterotrophic bacteria, the captured TSS which form a major portion of their food source, and--unfortunately--a lot of nitrifying bacteria. If the interval between backwashings is too short, the nitrifying bacteria will not have time to re-establish their population and a gradual wash-out will occur, dramatically limiting the nitrification capacity of the filter. The latter situation is managed by controlling the "Mean Cell Residence Time" (or MCRT) of the biofilm. The MCRT of a bioclarifier must be above 3-4 days for high rate nitrification to be observed. In early work (Malone et al, 1993), the MCRT was estimated by the "Sludge Residence Time" (or SRT) which is an estimate of the average length of time the sludge (the mixture of biofloc and captured TSS) is in the filter. The SRT can be approximated by conducting a sequential backflushing study (Malone et al., 1993) which will enable determination of the sludge harvest fraction for a single wash to be established. The SRT can then be approximated by the following equation (Coffin, 1993):

$$SRT=1/(H_f * TBBW)$$

where H_f is the fraction of the sludge removed by a single backwash and TBBW is the time between backwashes (in days). In early filters (Chitta, 1993), which were predominantly propeller-washed with spherical beads, the SRT was controlled by the physical configuration of the filter that controlled the harvest fraction per wash and the management scheme that defined the backwash frequency.

USDA SBIR Phase I

Management of filters under the assumption that MCRT=SRT creates a paradox since the need for high MCRT implies a high SRT. In other words, any attempt to increase the nitrifying population (as reflected by X_n in Equation 2) also increases S, the mass of solids which produce ammonia as they decay. Thus, as Chitta (1993) found, extending SRT first increases, then decreases, the VNC of a bioclarifier. Raising the design loading for a bioclarifier (expressed in pounds of feed applied per cubic foot of beads per day or Lbs/ft^3-day) demands a higher VNC. However, accelerated washing strategies failed through falling MCRT's, and extended washing schemes were frustrated by the increased solids interference. The optimum backflush frequency for propeller-washed filters was found to be about 48 hours, which corresponds to an SRT of 4-6 days. In bioclarifier application, the design loading was set at a feed-equivalent loading of about 1 pound of feed per cubic foot of beads, with VNC values in the range of 5-10 gm-N/ft^3 being displayed, since in most applications the filters were supported by in-situ nitrification (Mia, 1996). Loadings in the range of 1.5-2.0 lbs feed/ft^3 -day have been sustained by only a few researchers or commercial operators.

It was recognized that development of bead filters which are capable of supporting a load of 2 pounds of feed per cubic foot reliably without special operating protocols would effectively cut biofiltration costs in half and further enhance the attractiveness of bead filtration for recirculating applications. When Phase I of this research effort was proposed, the use of tubular media was suggested as a solution to this paradox. The tubular media selected consisted of chopped hollow tubing with an diameter of 3-6 mm (OD). Biofloc grows on both the outer and inner surfaces of the tubing. During the washing sequence the biofloc growing on the inner surface is protected from the abrasion induced by the mixing operation. The tubes also display a much higher porosity (85% versus 35% for beads). This means that they induce less headloss and they can hold more sludge before hydraulic performance declines. The tubes had a low specific surface area (250 ft^2/ft^3 versus 400 ft^2/ft^3 for beads) , but, it was felt that the management benefits would outweigh this disadvantage. After all, preliminary studies conducted in small 1 ft^3 bubble-washed bead filters filled with 6 mm chopped tubes had demonstrated elevated areal conversion values for tubes over beads and superior performance on a volumetric basis for extended backwash times at high loading.

In summary, at the start of Phase I the carrying capacity of plastic beads appeared to be limited by two inherent characteristics: (1) the entire surface of the bead is exposed to abrasion during the mixing process; and (2) the low porosity of the bed limited the range of SRT values that can be used to enhance nitrification before hydraulic clogging of the bed occurred. The use of the tubular medium was thought capable of overcoming these limitations since the tubes provide protected surfaces and display very high porosities. The research team proposed to investigate the effects of combining the paddle-washed technology with a tubular medium to see if enhanced carrying capacities could be realized. It was believed that the combined effects of reducing the flotation energy and mixing energy, increasing porosity, and providing protected surface area for biofilm development would allow the new filters to operate with more stability at loading rates in excess of 2 pounds of feed per cubic foot of beads.

Before the SBIR project was awarded and work began, the proposed approach was seriously undermined. The first negative development was the result of preliminary work undertaken by Sastry (1996). This, when completed, clearly indicated that tubes were inferior to spherical beads in bubble-washed filters. The tubes did encourage growth on the protected interior surface, but, once filled with biofloc, they exhibited a dramatic collapse of effective surface area; and this induced a hysteresis effect in performance. So, although superior under moderate loading regimes (about 1 Lbs/ft^3-day) the tubes were totally incapable of sustaining 2 Lbs/ft^3-day. Peak VNC values observed before collapse were only about 6 gm-N/ft^3-day. Further, Sastry's tubular bed displayed a dramatic increase in the hydraulic conductivity. The bed was clearly not "clogged" at the point of failure. In contrast, the bubble-washed bead filter filled with spherical beads sustained VNR values of 10 gm-N/day and feed loadings of 2 Lbs/ft^3-day. The bead filter, however, had to be backwashed 5 times daily and was operated at high pressures (about 10 psi) to overcome chronic problems with loss of hydraulic conductivity. Consistent with earlier experience, hydraulic conductivity did seem to limit the filter which was observed to be sensitive to the delivered flowrate (Qr). This sensitivity was attributed to inadequate oxygen supply.

Secondly, the results from two separate studies performed by Armant Aquaculture and LSU researchers, which were undertaken with funding from the Department of Energy and The National Coastal Resources Research Institute, were coming to light. These projects were studying, in part, the possibility of enhancing nitrification through the separation of the linkage of MCRT and SRT by reducing the biofilm abrasion during the backwashing process. The biofloc was still harvested, but at a more controllable rate. This was done by using large mixing paddles and slow rotation speeds (20-30 rpm). Although showing an improvement, the new "paddle-washed" filter did not substantially improve VNC's. The buoyancy of the beads in 2-4 foot deep beds can still cause sufficient abrasion to shear off large amounts of the desired biofloc, and pressure buildups quickly limit flow delivery to the bed. Paddle-washed bead filters behaved much like propeller-washed filters when used with spherical beads.

Consequently, the particulars of the Phase I study were altered in that (1) a modified bead (Figure 2) was substituted for one of the tubular media in the testing program and (2) a

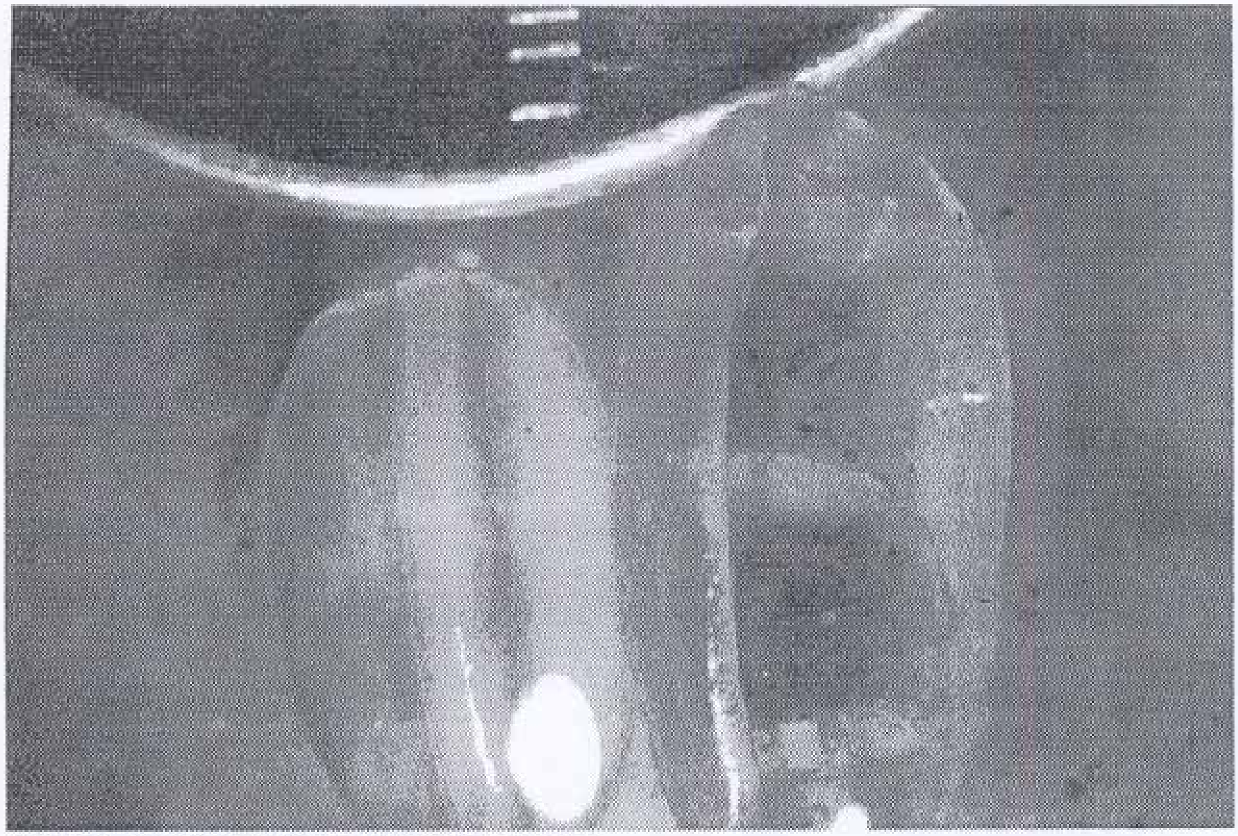

Figure 2. Modified bead used in SBIR Phase 1.

propeller washed platform was used (instead of the paddle-washed) for the testing program. Additionally, in cooperation with Armant Aquaculture, modified beads and tubes were placed in a number of prototype filters undergoing study at the University to broaden the base of experience.

The Phase I study involved a comparison of the standard low density polyethylene 1/8" feedstock, a feedstock bead "modified" by crushing, and 1/8" X 3/16" chopped straws (ACE-100) provided by Berg Bennett and Associates of Birchgrove Australia (Figure 3). The filters were attached to independently circulated 800 gallon tanks and subjected to comparable loadings. The findings showed that both the modified and tubular media displayed superior conversion as evidenced by the relative VNR values and tank concentrations (Figure 4).

Figure 3. Tubular media used in SBIR phase 1.

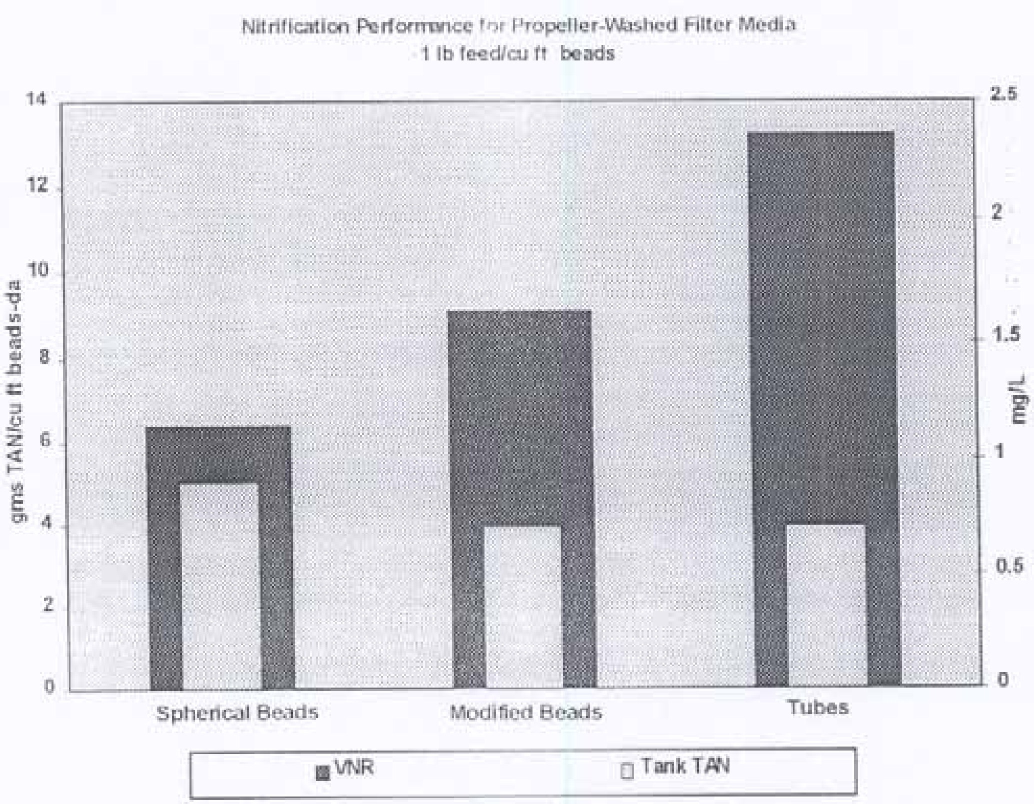

Figure 4. VNR and tank substrate concentrations for each bead media type with feed rate of 1 lb feed/ ft^3 bead media.

Additionally, the three cubic foot filters holding these beads were operated at substantially lower headlosses than the one holding the spherical beads. The basic observation was that at the loading regimes tested (up to 1 Lbs/ft^3-day), media with higher porosities and biofilm protection perform better in the propeller-washed filter platform. However, neither of the media-was
pushed to the failure point and the VNR's displayed averaged only about 9 mg-N/ft^3-day, falling well short of the value needed (About 15 to 20 mg-N/ft^3-day) to permit an increase in the filter carrying capacity to 2 Lbs/ft^3-day.

During the course of completing the Phase I project, the results from ongoing research at LSU have significantly clarified the difficulty associated with achieving the desired 2 Lbs/ft^3-day carrying capacity. First, work conducted by Golz (1996) revealed that two distinct classes of floating bead filters exist. "Aggressively washed" filters such as the propeller-washed filter (Chitta, 1993) and the paddle-washed filter show a high degree of biofilm abrasion during washing and therefore must be operated under the assumption that MCRT=SRT. The aggressively washed filters appear ultimately limited in carrying capacity (with spherical beads) by loss of hydraulic conductivity and by solids ammonification. To avoid the problems with pressure buildup, these filters are generally operated with backflush frequencies of once every two days or longer, a realm in which they are generally MCRT limited. The second classification, "gently-washed" filters, corresponding to the hydraulically washed unit of Wimberly (1990) and Sastry's (1996) bubble-washed unit, displays a high degree of biofilm protection for spherical beads and is virtually immune to MCRT problems. These filters generally display increasing VNR values as backwash frequency is improved. This permits the adverse impact of solids accumulation (ammonification and loss of hydraulic conductivity) to be virtually eliminated by high frequency washing as clearly demonstrated by Sastry's work. However, with spherical beads, Sastry was quickly approaching the point where simple porosity for storage of biofloc was limiting as was evidenced by the high back pressures associated with his tests.

The potential of combining modified (or tubular) media with high frequency washing is currently being demonstrated with some the modified beads supplied to LSU. Beecher et al. (1997) reports on current work funded by the Louisiana Sea grant Program with a bubble-washed bead filter containing our modified beads and reconfigured for airlift operation. The modified beads and a process control system allowed this filter to be operated at a high backwash frequency (12 times daily), which virtually eliminated the impact of solids accumulation. Testing of this filter at our design loading of 1 Lbs/ft^3 has displayed the very high VNR values (nearly 10 gm-N/-day as compared to 4 gm-n/ft^3-day by Sastry at the same loading). It is anticipated that this filter will be challenged to the failure point early this summer (1997). Although the VNR values surprised this SBIR research team, the low headlosses (about 0.5 cm/cm) displayed across the bed have allowed this filter to be operated with an airlift pump. Because of limited ability of the airlift to generate lift (about 30 cm at maximum), this filter test may not be able to demonstrate a bioclarification carrying capacity of 3 Lbs/ft^3. However, this airlifted, bubble-washed, bead filter's performance has already exceeded expectations and presents strong evidence in support of combining high frequency washing with bead modification as a means to increase carrying capacities.

Given the collective observations gathered over the course of the Phase I project, the SBIR research team's understanding of the problem has been clarified. The original proposition that the beads needed to be re-formed to protect bacteria has proved partially correct. It appears to hold true for aggressively washed filters, but not gently washed platforms. More importantly, porosity needs to be increased to provide more storage volume for the bacteria in a hydraulically uninhibited regime. It does us no good to hold bacteria if severe nutrient transport limits develop. Porosity may be the only parameter that is important in a gently washed regime. In an aggressively washed regime, adequate porosity as well as biofilm protection must be provided.

Secondly, the research team's perspective on the interference problem being caused by solids accumulation was naive. In retrospect, propeller-washed filter performance has been severely inhibited by secondary effects of solids accumulation. Previously, we only viewed the macro-scale problem, where loss of hydraulic conductivity caused by the mass of accumulated solids and the heterotrophic bacteria feeding on them inhibited advection in the bed to the point that oxygen limitation in the bulk liquid occurred. We can increase porosity to address the macro-scale transport problem, but this moves the transport limitation to the micro scale, i.e. diffusional constraints through the water boundary layer and biofilm. The breakdown rate of aquacultural solids is quick (Ning, 1996; Golz, 1996) and significant ammonia production and heterotrophic bacterial growth occurs. Development of thick biofilms dominated by heterotrophic bacteria will induce oxygen limitations within the biofilm. This is known to occur in domestic wastewater treatment where the TAN substrate levels are high (>5 mg-TAN/l) (Zhang et al., 1995). This reflects heterotrophic competition for oxygen within the biofilm, as well as constrained diffusion (Siegrist and Gujer, 1987).

In summary, recent research findings have led the research team to conclude that optimum nitrification performance in floating bead filters will only occur in filters subjected to high frequency washing, in which the adverse impacts of solids accumulation are virtually eliminated. The solids not only break down to produce ammonia (Matsuda et al., 1990), but also encourage rapid heterotrophic bacteria growth that competes with the nitrifiers for space, potentially limiting nutrients, and oxygen (Bovendeur et al, 1990). The beads will have to be re-formed to store increased volumes of nitrifying bacteria at loadings in excess of 2 Lbs/day-ft^3 without a reduction in hydraulic conductivity. The beads will also have to provide for abrasion protection in aggressively washed formats, but, excessive protection should be avoided.

(3) Relationship with Future Research or Research and Development

In the food fish area, there is much interest in the production of exotic or non-native species to serve local live or fresh fish markets (referred to as "niche marketing"). The most promising candidate to establish economic feasibility of recirculating technologies is clearly the warmwater tilapia, an exotic from Africa. Tilapia production facilities occur throughout the United States. Domestic tilapia production reached 12.5 million pounds (live weight) in 1993, up 40 percent from 1992 (ATA, 1994). ATA (1994) estimated that 1994 domestic production would reach 16.5 million pounds. Imports of tilapia also continued to increase at a rate of 50 percent per year to a live weight equivalent of over 32 million pounds in 1993 (ATA, 1994). Since they are warmwater fish, tilapia must be grown indoors for at least part of the year in most of the United States (USDA, 1994). The use of heat-conserving closed recirculating systems and/or utilization of geothermal or other low-cost heat sources has allowed production to expand to all regions of the country (USDA, 1994). The number of licenced producers in Louisiana, for example, has risen from 1 to 6 in recent years, despite the burdensome conditions imposed by strict exotic species regulations. Forecasted problems with disease and off-flavor in recirculating systems have not occurred in systems of modern design, which emphasize rapid sludge removal and maintenance of aerobic conditions. The market remains promising for live tilapia which demand approximately twice the price as catfish. A steady increase in the number and size of facilities is expected as the feasibility of the recirculating approach is clearly demonstrated in the commercial sector.

The ornamental fish trade is one of the fastest and most consistently expanding sectors of aquaculture in the U.S. In 1993, the U.S. exports of ornamental fish totaled $17.3 million, more than double the 1989 level (USDA, 1994), while imports of ornamentals in 1993 reached $45.2 million. The retail value of aquarium livestock sold annually in the U.S. has been estimated between $200 to $700 million (Winfree, 1989). Currently, 500 to 1000 species, including fresh and salt water fish, are sold for aquarium use. Of this number, about 80 percent of the trade volume is with species that can be farm-reared (Winfree, 1989). Adoption of recirculating system technologies for tropical fish production would facilitate the spread of the base of tropical fish production throughout the United States, and allow America to recapture some of the market from overseas sources. Also, an expanded domestic ornamental fish industry based on aquaculture would help to stem the over harvesting of ornamental fish and damage to delicate reef ecosystems that is reported to be occurring at a number of locations in southeastern Asia. Certain features of recirculating production systems, such as precise control over environmental factors, are particularly well suited to the needs of this developing industry (Weaver, 1991).

Another active research area has been in the use of recirculating systems to reduce heating costs associated with the production of aquatic reptiles, especially alligators. This industry has traditionally used high density culture in flow-through systems, usually batch discharges, and has incurred high labor and energy costs associated with the periodic manual cleaning of animal pits. Preliminary research results (Fall, 1993), indicate that significant gains in both areas can be achieved through extended re-use of culture waters. Crocodilian production is a growing industry worldwide (Zajicek, 1993). Since 1980, there has been an eleven-fold increase in the number of raw alligator skins exported from the U.S. (Zajicek, 1993). In 1990, Louisiana alligator farmers sold over $24.5 million worth of hides and meat (LCES, 1990). Today, however, market demand is soft and prices have fallen to a third of their peak value as competition has come from extensive pond production and wild harvests in developing and subtropical nations. A parallel industry based on the cultivation of freshwater turtles for food is likely to develop in future years (Culley and Falcon, 1991; Mayeaux et al., 1996).

There are several other applications of recirculating technologies with the potential to expand, given the proper impetus. These applications generally share characteristics that include rapid turnover of product and/or highly valued stocks that generate sufficient income to offset the cost of water reconditioning . Increasingly, as the demand for high quality seafood climbs, purging techniques are being used to enhance a variety of products. For example, purged crawfish which are held for 24 - 48 hours to remove external mud and gut contents, draw premium prices in local areas of production and are demanded in many parts of the country. Purging off-flavor from pond raised fish (Lelana, 1987) is routinely accomplished by holding unfed fish for 48 to 72 hours in clean waters (Yurkowski and Tabacheck, 1974). The value added and rapid turnover rates permit the economic use of recirculating systems for these purging functions. Often operated as open recirculating systems, these purging operations offer the users a high degree of control over product quality while minimizing source water problems and regulatory costs associated with water discharges.

One of the baseline requirements for a healthy aquaculture industry is a source of large numbers of disease free fingerlings (finfish), seed (clams), spat (oysters), or larvae (shrimp). Their weight-normalized value can be very high; thus the added expense of water reconditioning is affordable. Additionally, as improved genetic stocks are developed, valuable broodstock must be maintained and conditioned. The added protection provided by recirculating systems will offset their cost.

Another potential niche for recirculating technology is in the support of extensive pond culture operations through "head starting". This approach utilizes heated indoor systems to support fingerling growth early in the spring or late winter so that larger fish can be stocked in the ponds once the weather permits. Recirculating systems are extremely effective at conserving heat, and thus minimizing heating costs. Heated recirculating systems for increasing the stocking size of fingerlings can be used to enhance pond production rates (Losordo et al., 1992) or to extend pond culture techniques to higher latitudes. In combination with environmental controls to induce off-season spawning (Drapcho et al. 1987; Lawson et al. 1989), significant increases in production of red drum may be realized by growing the fry indoors under warm conditions during the winter months.

Sophisticated central water reconditioning systems are being utilized to reduce labor costs associated with the display and sale of ornamental fish (Weaver, 1991). It is not uncommon to see a retail tropical fish store link a few hundred tanks to a single filtration and disinfection unit. Additionally, an ever expanding potential use for recirculating systems is in live food fish display systems.

(4) Phase II Technical Objectives

The objectives of this research effort are to

1. Conduct pilot studies identifying the optimum filtration platform (propeller-washed, paddle-washed, or bubble-washed) for use with high-frequency washing of a selected modified media

2. Conduct pilot scale studies to determine nitrification and hydraulic performance characteristics of 1/8" d tubular, reformed, and extruded floating plastic media under a high frequency washing regime for the selected filtration platform.

3. Fabricate and evaluate a commercial scale (25-50 ft^3) airlift floating bead bioclarifier utilizing the selected modified plastic media and filtration platform.

4. Document capital, operational and energy savings associated with the frequently washed airlift-driven, modified bead filtration unit.

(5) Phase II Work Plan

<u>Task 1: Initial Reconfiguration of Pilot Scale Experimental System</u>

"CONFIDENTIAL PROPRIETARY INFORMATION"

Our consultant, Dr. Ronald Malone, (letter of support attached) will develop a design for the test units which will be specifically sized to operate with 3 cubic feet of beads, to allow full load testing in our test facility. This process involves determining the shape and dimensions of the filter hull, specifying inlet/outlet piping, designing the diffuser and paddle system, and sizing the mixing motor. Design drawings for the test units will be developed and delivered to AST. A second consultant, Mr. Vernon Rodrigue, Operations Manager of Vacherie Machine, will assist in the mechanical design and fabrication of specialized components not currently available in AST's inventory (see letter of support) .

The initial testing program will employ three 3 ft^3 bead filters, each configured to represent the mixing conditions associated with either (1) propeller-washed, (2) paddle-washed, or (3) bubble-washed platforms. One of the existing 3 ft^3 filters (Figure 5) can be utilized without modification. A second will be modified by Dr. Malone and Mr. Rodrigue to a paddle-washed configuration (Figure 6). The third will be a new configuration to be designated as a Marine Recirculating Bead Filter (MRBF). It will contain no metal parts and will feature low waterloss.

The 3 ft^3 MRBF prototype will be constructed from fiberglass, permitting easy fabrication without the use of expensive molds. This will involve (a) development of a filter hull geometry that is compatible with the proposed fabrication technique, (b) specification of inlet/outlet piping, and © design of the diffusers/screens which will be used to contain the media within the filter while allowing the sludge to freely discharge, as well as to resist clogging and biofouling.

The MRBF configuration presented in Figure 7 would operate normally in the filtration mode with water from a recirculating pump entering through the influent line, passing upwards through the static floating bead bed where the critical clarification and nitrification processes occur, and exiting through the effluent line before returning to the tank. The backwash pump is normally off, and seepage through this pump is prevented by a pressure release valve on its discharge side that is set to resist the normal range of backpressures encountered during the filtration cycle. The positive hull pressures keep the air inlet check valve closed. Any solids that remain in the settling chamber from a previous backwash event slowly settle into the sludge storage cone. A high-frequency backwashing MRBF can be expected to remain in the filtration mode for 1 to 6 hours between backwashes.

Backwashing would be initiated by a simple timer that deactivates the recirculation pump (not illustrated) and activates the backwash pump. The backwash pump will draw clean, settled water out of the filter casing above the sludge storage cone through an intake manifold, and the pump discharge head will open the pressure release valve. The ensuing head loss within the filter hull will allow the filter effluent check valve to close. This will, in turn, induce a sudden pressure drop within the filter hull that allows the air line check valve to open and admit a sudden influx of air. The air will rise up into the filtration chamber, creating turbulence in the water that supports the floating bead medium. Beads dislodged by this turbulence will then drop downwards towards the washing throat. A plastic screen in the washing throat will allow the released solids to pass downward into the settling chamber while preventing escape of the beads. The backwashing event duration will be timed (<60 seconds) so that waters dirtied by

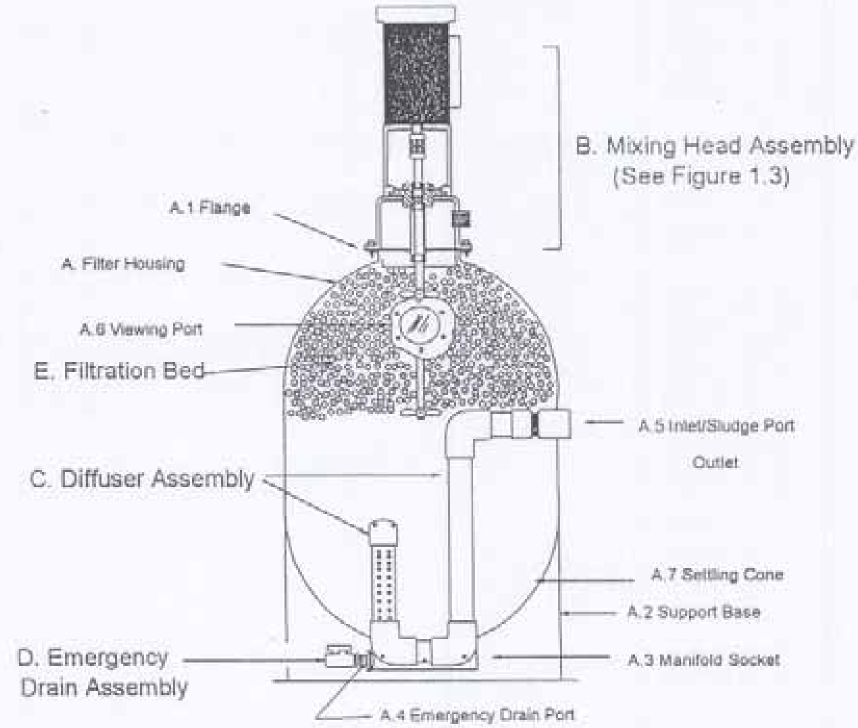

Figure 5. Standard Propeller-Washed Filter Configuration

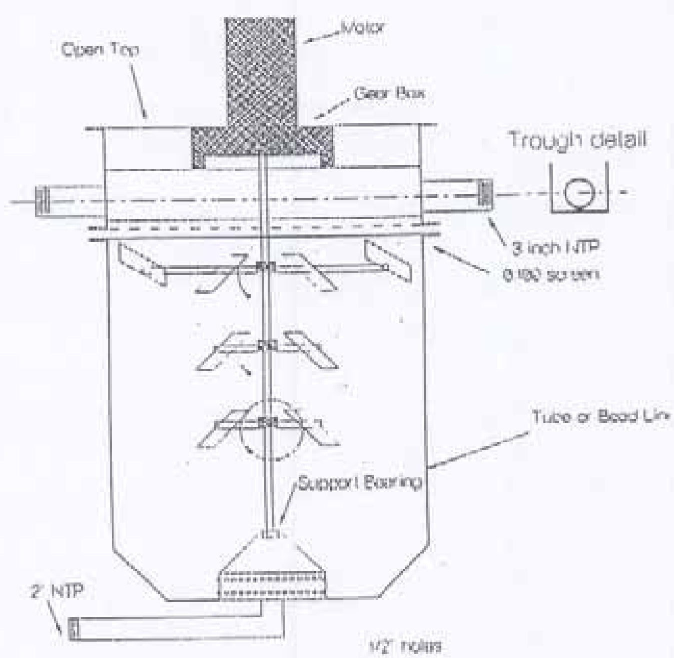

Figure 6. Paddle Washed Filter Configuration.

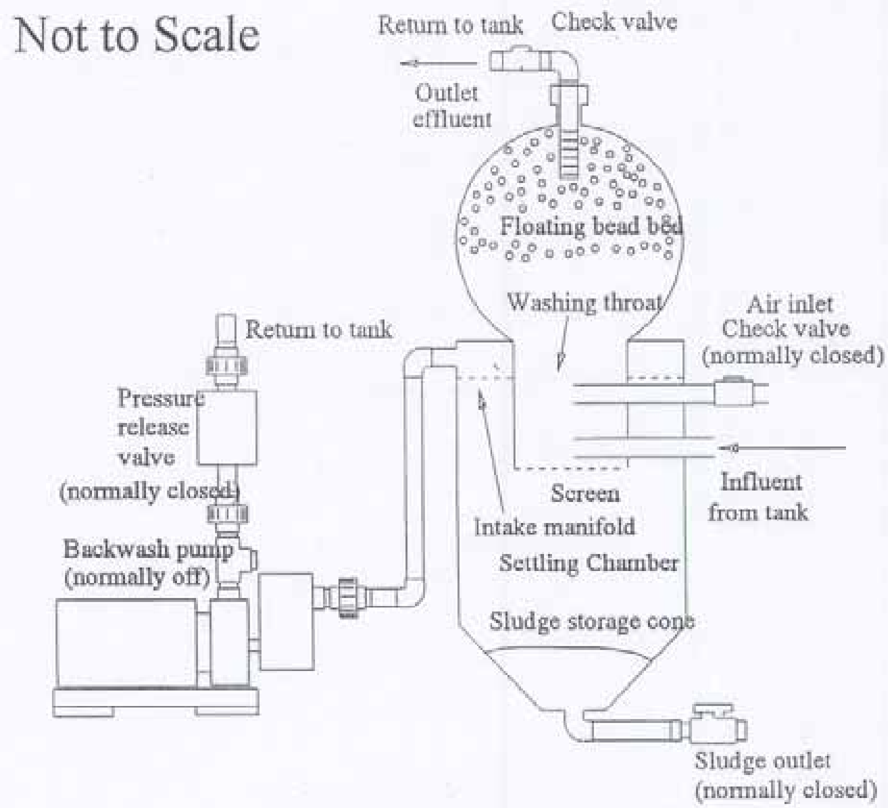

Figure 7: The envisioned Marine Recirculating Bead Filter (MRBF) Configuration.

the washing action cannot reach the backwashing pump inlet manifold. The clear water picked up by the backflushing pump will be directed back to the recirculation system. The water captured in the settling chamber will then be slowly clarified during the next ensuing filtration cycle.

While backwashing can be effectuated as often as every 30 minutes, sludge removal via the storage cone can be undertaken at greatly extended intervals, perhaps as long as a week, depending on the volume of sludge storage. Sludge can be drawn off at any time by manually opening the sludge valve for about a minute.

The MRBF schematic (Figure 7) is not drawn to scale and is meant for illustrative purposes only. The unit is designed to overcome the high water loss rates associated with implementation of a high frequency washing regime in an hour-glass format. Successful implementation of this filtration strategy is dependent upon timing of the backwash pump's operation, proper hydraulic design, and proper proportioning of the various components.

The existing three replicate 800 gallon recirculating systems will be used for the experiments. An additional three 1000 gallon polytanks will be purchased to assure that we have enough tank volume to support our peak tests. Each system will consist of a circular eight hundred gallon polyethylene tank, a 1 horsepower centrifugal pump, the prototype bead filter, a foam fractionator, and an aeration system consisting of a distribution manifold with 24 eight-inch, airstones. Air will be delivered to the three systems from two centralized 2.5 horsepower rotary blowers. The aeration system is oversized to provided redundancy for the critical delivery of oxygen while facilitating degasification (carbon dioxide stripping) during normal operation. The facility will be equipped with a telephone based alarm system and a backup diesel powered aerator to assure the survival of stock during power failures.

Although the research effort is directed towards the development of an airlift configuration, these preliminary tests will be conducted with centrifugal pumps to permit a wide range of headloss conditions to be readily investigated. Each filter will be equipped with Pitot tubes and pressure gauges that will permit a measurement of headloss across the bed in the range of a few inches to several psi. The media used in the initial test will be the KMT-Biofilm Carrier Elements originally developed by the Norwegian research Institute SINTEF. This media (Figure 8) provides both internal and external surface area protection and its use in this phase of the experimental regime will allow the research team to examine the relative protection provided to these distinctly different biofilm growth zones.

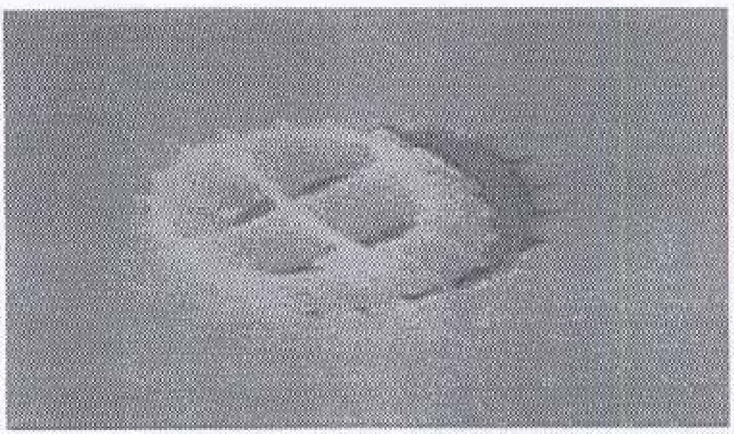

Figure 8. KTM-Biofilm Carrier Elements Developed By the Norwegian Research Institute SINTEF.

Task 2: Modification of Backwashing control boxes

The potential benefits of process control to the aquaculture industry have been described in the literature (Lee, 1991; Lee, 1993). Implementation of a high frequency washing strategy will require electronic control of the washing sequence. Controller boxes will be modified as necessary from present controllers developed during Phase I . These controllers contain a field programmable microcomputer (MPU) and a central processing unit with both random access and read only memory (RAM and ROM). The current propeller-washed units provide for 16 interval and 16 duration settings, permitting the implementation of a wide variety of backwashing regimes. Four internal Opto 22 optically coupled solid state relays perform timed off/on operations implementing control of the circulation pump, the mixing motor, and the opening/closing of actuated ball valves (ASAHI Electromni Actuated Ball devices are frequently used). The unit also includes a the pressure driven microswitch which can be utilized to trigger the backwash frequency in response to pressure buildup beneath the filtration bed.

The manufacturer of our controllers, Innovative Environmental Solutions, LLC, (see letter of support) has agreed to work with AST to make the modifications necessary to the current controller to allow us to implement the research plan.

Task 3: Acclimation to Peak Loading Conditions

Development of a biofilm layer on the media is required for biofiltration. Initially the biofilter has no bacteria and the culture must be started. The process of growing the initial bacterial culture in the biofilter or adjusting an established culture to a change in loading is called acclimation. The three prototype filters will be acclimated by heavily seeding them with backwash waters from bead filters at the TilTech facility. Nitrite at a level of one mg-N/l will be dosed to provide an initial substrate for the Nitrobacter sp. to avoid delays in initiating this slow growing genus of bacteria (Manthe and Malone, 1987). Backflushing will initially be set at a low frequency to minimize biofloc loss during this critical start-up period. The nitrifying bacteria are very slow reproducers and for this project thirty days of acclimation with feeding will be provided (2 - 3 weeks is typical) to fully establish the bacterial culture.

Once initial acclimation is achieved, fish will be added incrementally and the feeding levels in the systems will be gradually increased. To avoid harming the fish, density adjustments will only be made when both TAN and nitrite-nitrogen levels are below one mg-N/l. Additionally, chloride (by $CaCl_2$ addition) levels will be raised and pH levels moderated (to about 7.2) to reduce the sensitivity of the fish to nitrites and TAN, respectively. The backwash frequency will be increased with the feed loading. The pH will be increased to the optimum range for the tests. This process will be continued until a feed application rate of at least three pounds per day is achieved in each system, representing the 1 Lbs/day design criterion for finfish growout. We anticipate that about two months will be needed for this initial acclimation, debugging and loading phase.

The organisms used to generate the waste loading for the prototype testing program will be ornamental Koi carp culls (*Cyprinus carpio*). The experiments will be conducted in a manner that assures that peak TAN and nitrite values will remain below 5 mg-N/l. Corrective action will be taken whenever a threshold of 1.0 mg-N/l for either parameter is reached. Koi are generally hardy and will display no adverse impacts to the TAN and nitrite-nitrogen at the levels maintained during the testing. At the end of the experiments the fish will be returned to LSU for their testing programs or donated to commercial tilapia producers in the area that utilize the fish as "sweepers" for the bottoms of their tilapia tanks.

Task 4: Stress Testing of Filtration Platforms

Flow rates through the filters will be set at an equivalent of 10 gpm/ft^3 or 30 gpm for each filter and maintained at this rate for the duration of the study. Alkalinity will be maintained above 100 mg/l-$CACO_3$ with the periodic addition of sodium bicarbonate (baking soda). The pH will be maintained between 7.5 an 8.0 by manipulation of the aeration/degasification systems (Grace and Piedrahita 1994; Allain 1988; Loyless 1995).

The experimental testing regime will be consist of three one-month experiments. The first, initiated just after filter acclimation, will subject the three prototypes to waste loadings corresponding to the current design standard of one pound of feed per cubic foot of media per day (1 lb/ft^3-day). Each system will, therefore, receive three pounds of feed per day. During this initial evaluation run the response of the system to variations in the washing procedure will be examined to establish a near-optimal operational regime that can be used to tackle the heavier loading regimes in the second and third experiments. The second one month study will be conducted at a loading rate of 2.0 lb/ft^3, or 6.0 pounds of feed per system each day, and the third at the desired 3.0 lb/ft^3, or 9.0 pounds of feed per system per day.

All experimental runs will be conducted under similar environmental and water quality conditions. All loading experiments will be conducted with a 37% protein catfish feed. Each prototype tank will be stocked with Koi at a sufficient density (up to 900 pounds per tank) to assure that all the feed added to the system is consumed. Fish will be fed three times a day to assure a representative temporal variation in waste generation. Temperature will be maintained at $30^0 \pm 1^0$ Celsius for the duration of the experiments. Dissolved oxygen levels in the tank will be held above 5 mg-N/l while filter effluent dissolved oxygen levels will be maintained above 2 mg/l. Flow rates to the filter will be set initially at thirty gallons per minute and adjusted whenever water quality conditions dictate. Alkalinity will be maintained above 100 mg-$CaCO_3$/l and pH maintained between 7.5 an 8.0 by manipulations of the aeration/degasification system.

All the prototype systems will be monitored daily for feed consumption, water flowrates, airflow rates, pH, temperature, backpressures on the influent side of the filter, in-tank dissolved oxygen levels, and filter effluent dissolved oxygen levels. TAN and nitrite levels will be monitored daily with small aquarium indicator kits to assure that water quality is suitable for the fish. Once a steady state performance level is reached (i.e. the system adjusts to a feeding rate increment), three sets of filter performance data consisting of both influent and effluent samples for TAN, nitrite-nitrogen, headloss across te bed, flow rates, total suspended solids, dissolved oxygen, Ph, alkalinity, five-day biochemical oxygen demand, and turbidity will be collected and sent to LSU for analysis.

Task 5: Water Quality Analysis

Water quality analysis will be conducted by the CEASL water quality lab in the Department of Civil and Environmental Engineering at Louisiana State University (see letter of support from Ms. Christy Higginbotham). All samples will be analyzed according to accepted methods (TABLE 1) outlined in Standard Methods (APHA, 1995) . The laboratory will supply AST with a written report of results. All water quality samples taken from the pilot scale system will be analyzed in triplicate and reported as mean ± std deviation. These results will be used to guide adjustments in management strategies and will serve as the main basis for analysis.

Task 6: Interim Analysis of Water Quality Results

The data collected will be converted to standard measures of biofilm performance. The areal ammonia conversion rate constant, C_A, is the traditional measure of how effectively a biofilter converts ammonia to nitrite (Malone et al, 1993). The units of C_A are milligrams TAN converted per square foot of media area per day (or mg/ft^2-day). It is computed in a manner similar to the VNR (Equation 1):

The third equation which proves very helpful in the management of bead filters measures the amount of bacterial action by calculating the areal oxygen consumption rate, C_{XT}:

$$C_{XT} = \frac{5{,}450\ (O_I - O_E)\ Q}{(V)\ (S_A)} \tag{5}$$

where O_I is the influent dissolved oxygen concentration (mg-O_2/l) and O_E is the dissolved oxygen concentration (mg-O_2/l) in the water leaving the filter. C_{XT} measures the combined respiration activity of the nitrifying bacteria, the heterotrophic bacteria extracting soluble BOD from the water column, and the heterotrophic bacteria responsible for the breakdown of solids (sludge) held in the filter. The apparent areal oxygen consumption rate C_{XN} (mg-O_2/ft^2-day) of the nitrifying bacteria can be computed directly from the areal conversion rates for nitrification since we know the amount of oxygen required for nitrification from chemical equations:

$$C_{XN} = 3.43\ C_A + 1.14\ C_N \tag{6}$$

The areal oxygen consumption rate, C_{XH} (mg-O_2/ft^2-day) which can be attributed to heterotrophic activity can then be calculated by the difference:

$$C_{XH} = C_{XT} - C_{XN} \tag{7}$$

Finally, the percentage of respirational activities that can be attributed to nitrification, F_{XN}, can be defined:

$$F_{XN} = C_{XN}/C_{XT} \cdot 100 \tag{8}$$

Generally, optimum filter performance (high C_A and C_N) is associated with moderate to low values of C_{XT} and thus high values for F_{XN}. The magnitude of C_{XT} generally increases with increases in organic loading (feed rate) and declines with increasing backflushing regime.

The primary criterion for selecting the best filtration platform will be the ratio of the estimated cost of ownership (\$/ft^3-day) to the volumetric nitrification rate (gm-N/ft^3-day) which defines the cost of converting a gram of TAN to nitrite. Additional consideration will be given to the residual substrate concentrations observed in the systems (TAN_I & N_I), as well as, the feed loading rate. The areal conversion/consumption rates will be used as interpretive tools as they are closely related to the condition of the biofilm and can be most closely related to issues such as biofilm thickness, heterotroph/nitrifyers ratio, and diffusion. All comparisons will be made with appropriate statistical techniques.

Task 7: Stage II Pilot Scale System Reconfiguration

The best filtration platform will be selected from data generated under Task 5. The other two candidate filters will be replaced with units identical to the one selected. The three filters will then be filled with new media. The media (the modified beads and the ACE-100) which performed well during the Phase 1 experiment A will be used to challenge the KMT-biofilm carrier elements during this experiment. The modified beads provide external protection only, the ACE-100 internal protection only, and the KMT-biofilm carrier elements provide both internal and external protection, so this comparison should allow the research team to answer some fundamental questions.

Task 8: Stage II Acclimation to Peak Loading Conditions

The acclimation process described under Task 3 will be repeated for the three filters and the systems gradually brought to design loading over about a two-month period. All three filters will be subjected to identical high frequency backwashing regimes with continued monitoring of headloss to assure that the filters are operated in a manner consistent with airlift operation.

Task 9: Stage II Stress Testing of Media

The three filters will again be subjected to an increasing loading regime to determine which of the media best supports the feed loadings of 1, 2 and 3 Lbs/ft^3. The systems will be sampled in triplicate as they reach steady state at each loading. Headloss and flowrate will be sampled to assure that sufficient data for the hydraulic analysis is obtained.

Task 10: Analysis of Results from Stage II Testings

The data collected under Task 8 will be reduced to basic volumetric and areal engineering parameters as described previously under Task 6.

TASK 11: Hydraulic Analysis

A detailed analysis of the hydraulic performance of the best media/filter platform combination selected is critical for the successful design of an airlift configuration for the commercial testing program. Of critical importance is the relationship between the recirculating flowrate and the headloss through the media bed. Airlift pumps are most economical when they are used to lift the water only a short distance. We will attempt to enhance a filter design that will operate at peak loading with a total lift of less than 2 feet.

Hydraulics through the bead bed can be described through transport phenomena in porous media, which are extensively treated in the literature (Cunningham, 1991; Kovacs, 1981; Halek and Svec, 1979; Scheidegger and Liao., 1972). Cunningham et al. (1991) summarized the equatiic characteristics of porous media with accumulated biofilm. Equation 4 below (Darcy's law) describes the relationship between velocity and piezometric gradient (dh/dL). The coefficient K is called the hydraulic conductivity, which is a measure of the conductance of the porous media to fluid flow. K depends on the properties of both the media and the fluid, and in the bead filter, will be greatly affected by biofilm development.

$$V = K\frac{dh}{dL} \tag{9}$$

where:

V = specific discharge or fluid superficial velocity (mm/sec)
K = hydraulic conductivity (mm/sec)
dh/dL = piezometric gradient (unitless) = H/L

H = headloss (m)
L = flow path along which the headloss H occurs (m).

The Carmen-Kozeny relationship, can be used to relate headloss to the porous media porosity and friction (f) as shown in Equation 10. Equation 11, the Carmen-Kozeny theoretical equation for estimating porous media friction factor (Cunningham et al., 1991), in turn provides the relationship between f and α.

$$H=\frac{f(1-\alpha)LV^2}{\Theta\alpha^3 dg} \tag{10}$$

$$f=\frac{150(1-\alpha)+1.75}{N_R} \tag{11}$$

where:

f = porous media friction factor (unitless)
α = porosity (unitless)
Θ = shape factor (a measure of the deviation of the particle's shape from that of a perfect sphere; usually assumed as 1).
g = gravitational constant (9.8 m/sec^2)
d = diameter of media particles (mm)
N_R=Reynold's number

Reynold's number is a widely used measure of turbulence and can be defined for a porous media by Equation 12.

$$N_R=\frac{Vd}{\nu} \tag{12}$$

where:

ν = kinematic viscosity (m^2/sec).

As examination of Equations 9-12 through 7 indicates, buildup of headloss through a porous bioclarifier bed is complex. As the biofilm thickens, the effective diameter of the beads increases and the effective porosity decreases. This causes a buildup of head, which in the case of an airlift system generally results in a declining flow rate through the bed. So, the head loss flow relationships have to be developed for specific design loadings under a prescribed backwash frequency. The bead design must accommodate the required mass of biofilm while maintaining enough porosity to deliver the minimum flow rate required for oxygen delivery within the headloss constraints imposed by the airlift system. Another major design variable is the cross-sectional area of the bead, which dramatically affects the headloss by changing the superficial velocity (V) and the bed depth (L).

The situation is further complicated by the hydraulic characteristics of the airlift pump. The airlift pump in a bead filter is normally placed on the effluent side of the filter to avoid the disrupting effects of small micro bubbles generated by the air injection process. Increases in headloss through the bed then induce an increase in the lift required, as well as a loss in effective submergence depth, which further contributes to a loss in pumping efficiency.

Equations 9 through 12 will be applied to the data collected from the prototype testing experiments (Tasks 4 and 9) to determine the values for the coefficients required to develop headloss versus flow relationships for projected loading schemes. These results will then be coupled with relationships defining the flow delivery rates for airlift pumps. This will allow the analysis of the various possible combinations of bed depth, flowrates, depths of submergence, and head loss.

TASK 11: Development of Commercial Scale Design

AST's consultant, Dr. Ronald Malone, with the assistance of Mr. Rodrigue, will utilize the results of the analysis conducted under Tasks 9 and 10 in the design of a commercial prototype with a modified bead volume of about 25 ft^3. This filter will be assumed to have a design carrying capacity of 2 Lbs/ft^3 which means that it should be capable of supporting about 5,000 lb of foodfish at its design level. This unit and the associated airlift pumping system will be designed to accommodate a flowrate required for oxygen delivery (150-200 gpm) at an operational headloss of 12-24 inches under a high frequency washing regime.

Task 12: Commercial Prototype Production

The design generated under Task 11 will be constructed by a fabrication process that will start with the development of a mold for a polyethylene tank that will form the hull of the bioclarifier. The mold will be fabricated with the assistance of personnel from Rhino Roto Molding, Maple Lake, Minnesota (see letter of support). AST has found the polyethylene molding costs and an initial prototype production run to be less costly than the more traditional approach of developing fiberglass molds and glassing the initial prototype.

The AST staff will complete fabrication of the prototype. Mr. Rodrigue will supervise the fabrication to make sure that the unit is adequately constructed to withstand operational stresses. Of particular concern in this regard, should a mechanical mixing platform be selected, is the transfer of torque from the mixing head to the polyethylene shell.

Task 13: Installation of Commercial Prototype

The AST staff will install the prototype, associated air blower, and electronic control boxes at the TilTech tilapia facility in Robert, Louisiana (see letter of support, Appendix C), an hour's drive from our office. This process will require (a) digging a hole to bury the unit adjacent to the in-ground rearing tank; and (b) on-site fabrication of the siphoning and airlift apparatus. The unit will be connected to a 40,000 gallon production tank, which will have the aeration and degasification capacity to support at least 10,000 pounds of tilapia with a peak feeding rate of about 120 lb per day. TilTech Mr. Steve Abernathy, the owner of the TilTech facility and his staff will be given a brief training program on the operation of the filter. The AST staff will closely monitor the unit through the debugging stage to assure that all components including the electronic control boxes are operating properly.

Task 13: Stage III Demonstration in Commercial Facility

The TilTech staff will assume all day to day operating responsibility for the test system. AST staff members will monitor the unit approximately twice a week during the test period. Otherwise, the test unit will be treated by the TilTech staff as part of the normal production system. The TilTech facility currently operates entirely with floating bead filter bioclarifiers, so the staff is already highly qualified to operate the unit. They have a track record of operating the earlier bead filter models at about twice the recommended design capacity. The units will be fed several times daily by hand. Mr. Abernathy will increase the fish loading and feed rate incrementally until the filter fails. Failure is generally indicated by a reduction in feed consumption, and a corresponding elevation of TAN levels above 1 mg/l. The feed level will then be reduced to a safe sustainable level and maintained there to maximize production. Data collected from the unit will be immediately reviewed by Dr. Malone, and modifications made to the operational regime to increase the carrying capacity. Modifications may include changes in the backwash frequency, duration of wash, settling times, or air distributions (between the airlift pump and the airstones). The TilTech staff will continually challenge the carrying capacity limits with hand feeding and observation techniques.

Once acclimation of the filter occurs in the commercial facility, intensive sampling every other week will be initiated. A complete filter performance data set consisting of influent and effluent samples for TAN, nitrite-nitrogen, flowrate, headloss measures, air delivery amounts, total suspended solids (TSS), dissolved oxygen, pH, alkalinity, five-day biochemical oxygen demand (BOD_5) and turbidity will be collected by AST personnel and transported to the water quality laboratory at LSU for analysis (Task 5). These data will be collected routinely without regard to the loading regime or management routine. The evaluation will be conducted for a six-month period.

Task 14: Analysis of Stage III Results

The data collected under Task 13 will be integrated with the findings generated from the two earlier experimental runs and analyzed with the principal objective of determining a safe design capacity in terms of lb-feed/ft^3-beads per day. This will require an examination of the relationship between VNR and residual substrate levels (TAN and Nitrite) during both the commercial and the experimental evaluation programs. Recommendations for carrying capacity and operational guidelines for the prototype configurations will be developed and documented in a short report.

Task 15: Economic Evaluation

Costs will be delineated and compared, category by category, against the current bead filter designs. Overall costs will be normalized on the basis of carrying capacity. The economic evaluation will be based on consideration of ownership (capital and operations costs) over a ten year design life. The results will be expressed in terms of cost of ownership per pound of feed supported per day and per Kg of TAN converted per day. These costs will be compared to the cost of ownership for existing AST models of similar capacity and for competing technologies to determine the economic feasibility of initiating commercial production of a full line of airlift filters employing scale tube filters.

References

ATA. (1994) *Annual Tilapia Situation and Outlook Report.* American Tilapia Association.

Ahmed, H. U. 1996 The Effects of Fluxrate and Solids Accumulation on Small Size Particle Accumulation in Expandable Granular Bead Filters. M. S. Thesis, Louisiana State University, Baton Rouge, LA

Allain, P. A. 1988. *Ion Shifts and pH Management in High Density Shedding Systems for Blue Crabs (Callinectus Sapidus) and Red Swamp Crawfish (Procambarus Clarkii)*, Master's Thesis, Louisiana State University, Baton Rouge, Louisiana, 77 pp.

APHA. 1995. *Standard Methods for the Examination of Water and Wastewater.* 19th edition, American Public Health Association, New York.

Beecher, L. E., A. A. DeLosReyes. R.F. Malone. 1997. Evaluation of an Air-Driven Recirculating System Employing a Bubble-Washed Bead Filter. Presentation at World Aquaculture Society Annual Meeting. Seattle, Washington. Feb 18-24.

Bovendeur, J., Zwaga, A. B., Lobee, B. G. J., and Blom, J. H. 1990. Fixed-biofilm Reactors in Aquacultural Water Recycle Systems: Effect of Organic Matter Elimination on Nitrification Kinetics. Water Research, Vol. 24, No. 2, pp. 207-213.

Chitta, B. S. 1993. Effects of Backwash Frequency on the Nitrification in Plastic Bead Media Biofilters Used in Recirculating Finfish Culture Systems. M. S. Thesis, Louisiana State University, Baton Rouge, Louisiana.

Coffin, D. E. 1993. Impact of Solids Retention Time on Sludge from Low Density Media Expandable Granular Biofilters, M.S. Thesis, Louisiana State University, Baton Rouge, LA

Cooley, P. E. 1979. Nitrification of Fish-Hatchery Reuse Water Utilizing Low-Density Polyethylene Beads as a Fixed-Film Media Type. M. S. Thesis, University of Idaho, Moscow, Idaho.

Culley, D. D., and G. M. Falcon. 1991. Growth, Feed Consumption and Feed Conversion of Common Snapping Turtles on Two Diets. Proc. Conference on Nutrition of Captive Wild Animals , Chicago, IL. December 13, 1991.

Cunningham, A. B., W. G. Characklis, F. Abedeen, and D. Crawford. 1991. Influence of Biofilm Accumulation on Porous Media Hydrodynamics. Environmental Science Technology 25: 1305-1311.

Dicks, M. and Harvey, D. October, 1988. Aquaculture Situation and Outlook. U.S. Department of Agriculture Economic Research Service. Aqua-1.

Fall, L. 1993. Water Recirculating Systems for Alligators. Proc. LA Aquaculture Conference, Baton Rouge, LA. pages 50-60.

Drapcho, C., MacNamara, T. B. Lawson, T. B. Braud, and W. R. Wolters. 1987. ASAE Proceedings of the 1987 Summer Meeting. Paper No. 87-5033.

Hopkins, T. A. and W. E. Manci. 1989. Feed conversion, waste and sustainable aquaculture, the fate of the feed. *Aquaculture Magazine*, 15(2):30, 32-36.

Golz W., K. A. Rusch, & R. F. Malone. 1996. Developing Backwash Protocols for Floating Bead Filters : A Model of Solids-Loading and Biofilm-Retention Effects on Nitrification. Aquacultural Engineering Society Proceedings II, Successes and Failures in Commercial Recirculating Aquaculture, a Publication of the Northeast Regional Aquaculture Engineering Service (NRAES-98, Volume 1:196-205)

Grace, G. R. And R. H. Piedrahita. 1994. Carbon Dioxide Control, In Aquaculture Water Reuse Systems: Engineering Design and Management, M. B. Timmons and T. M. Losordo (eds), Elsevier Science Publishing Company. Amsterdam, The Netherlands. pp. 209-234

Halek, V. and J. Svec. 1979. Groundwater hydraulics. Elsevier Scientific Publishing Company, Amsterdam.

Huguenin, J. E. and Colt, J. (1989) Design and Operating Guide for Aquaculture Seawater Systems, Developments in Aquaculture and Fisheries Science, Vol. 20, Elsevier, 264 pp.

Joint Subcommittee on Aquaculture. 1993. Aquaculture in the United States: Status, opportunities and recommendations. A Report to the Federal Coordinating Council on Science, Engineering, and Technology. p 21.

Kovacs, G. 1981. Seepage hydraulics. Elsevier Scientific Publishing Company, Amsterdam.

Lawson, T. B. 1994. Fundamentals of Aquacultural Engineering. Chapman & Hall, ITP Publishing Company, NY, 355 pp.

Lawson, T. B., C. M. Drapcho, S. MacNamara, H. J. Braud, and W. R. Wolters. 1989. Aquacultural Engineering, Vol. 8:177-191.

Lelana, I. Y. B. 1987. Geosmin and Off-Flavor in Channel Catfish, Ph.D. Dissertation, Auburn University, Auburn, Alabama.

Lee, P. G. (1991) Automation of Aquaculture Systems: A review and Practical Guide to Implementation. Proc. of Symposium on Engineering Aspects of Intensive Aquaculture. Northeast Aquaculture Engineering Service, Ithaca, NY. Pages 284-300.

Lee, P. G. (1993) Computer Automation for Recirculating System. In: Techniques for Modern Aquaculture. J.K. Wong (ed.), American Society of Agricultural Engineers, St. Joseph, MN, pp. 61-70.

Losordo, T. M. and Westerman, P. W. "An Analysis of Biological, Economic, and Engineering Factors Affecting the Cost of Fish Production in Recirculating Aquaculture Systems," pp. 1-9 In Workshop Proceedings on Design of High Density Recirculating Aquaculture Systems, 1993, Louisiana State University, Baton Rouge, Louisiana, September 25-27, 1991.

Losordo, T.M., M.P. Masser, and J.E. Rakocy. 1992. Recirculating aquaculture tank production systems: an overview of critical considerations, Southern Regional Aquaculture Center. Publication 451.

Loyless, J. C. 1995. "A Feasibility Study of Using Air-lift Pumps for Aeration, Degasification and Water Movement in a Recirculating Aquaculture System". Master's Thesis, Louisiana State University, Baton Rouge, Louisiana 116 pp

Malone, R. F., B. S. Chitta, and D. G. Drennan. 1993. Optimizing Nitrification in Bead Filters for Warmwater Recirculating Aquaculture Systems. In Techniques for Modern Aquaculture, edited by Jaw-Kai Wang. ASAE Publication 02-93 (ISBN 0-9293355-40-7;LCCN 93-71584).

Malone, R. F. and Coffin, D. E. "Biofiltration and Solids Capture with Low Density Bead Filters," pp. 29-35, In Workshop Proceedings on Design of High Density Recirculating Aquaculture Systems, 1993, Louisiana State University, Baton Rouge, Louisiana, September 25-27, 1991.

Malone, R. F. 1992. Floating Media Biofilter, U.S. Patent No. 5,126,042, June 30.

Malone, R.F. 1993. Floating Media Hourglass Biofilter, U.S. Patent No. 5,232,586, August 3.
Malone, R. F. 1995. Floating Media Biofilter, U.S. Patent No. 5,445,740. August 29

Manthe, D. P., and R. F. Malone. 1987. Chemical Addition for Accelerated Biological Filter Acclimation in Closed Blue Crab Shedding Systems, Aquacultural Engineering, 6:227-236.

Manthe, D. P., R. F. Malone, and S. Kumar. 1988. Submerged Rock Filter Evaluation Using an Oxygen Consumption Criterion for Closed Recirculating System. Aquacultural Engineering, 7: 97-111.

Matsuda, A. T. Ide, & S. Fuji. 1988. Behavior of Nitrogen and Phosphorus During Batch Aerobic Digestion of Waste Activated Sludge-Continuous Aeration and Intermittent Aeration by Control of DO. Water Research 22:1495-1501.

Mayeaux, M. H., D. D. Culley, and R. C. Reigh. 1996. Effects of Dietary Energy: Protein Ratio and Stocking Density on Growth and Survival of Common Snapping Turtle, *Chelydra serpentina*, J of World Aquaculture Society. Vol 27, No.1 (March).

Mia, R. I. 1996. In Situ Nitrification in High Density Recirculating Aquaculture Production Systems. M. S Thesis, Louisiana State University, Baton Rouge, Louisiana.

Nash, C. J., 1988. *Journal of World Aquaculture Society*, 19: 51.

Ning, Zheng. 1996. Characterization and Digestibility of Aquacultural Sludge. Master's Thesis, Department of Civil and Environmental Engineering, Louisiana State University, Baton Rouge, Louisiana, 70803.

O'Rourke, P. D., "Preproduction Analysis of Economic Profitability for Recirculating Aquaculture Systems," pp. 16-17 In Proceedings Workshop on Design of High Density Recirculating Aquaculture Systems, 1993, Louisiana State University, Baton Rouge, Louisiana, September 25-27, 1991.

Sastry, B. N. 1996. A Comparison of Nitrification Capacity in Bead and Tubular Plastic Media. M.S. Thesis, Louisiana State University, Baton Rouge, Louisiana.

Scheidegger, A. E. and K. H. Liao. 1972. Thermodynamic Analogy of Mass Transport Processes. IAHR (International Association for Hydraulic Research). Fundamentals of transport phenomena in porous media. Elsevier Publishing Company, Amsterdam.

Siegrist, H., and W. Gujer. 1987. Demonstration of Mass Transfer and pH Effects in a Nitrifying Biofilm. Water Research, 21(12): 1481-1487.

Timmons M.B., and T.M. Losordo (eds.). 1994. Aquaculture Water Reuse Systems: Engineering Design and Management. Developments in Aquaculture and Fisheries Science, 27. Elsevier, Amsterdam, xii + 333 pp.

USDA. (1994) *Aquaculture: Situation and Outlook Report.* United States Department of Agriculture. Publication AQS-12.

Weaver, D. E. 1991. Performance of Fine Sand Fluidized Bed, In Proceedings of Workshop on Design of High Density Recirculating Aquaculture Systems, 1993, originally presented at Louisiana State University, Baton Rouge, Louisiana, September 25-27, 1991. pp 18-23

Wheaton, F. W. (1985) Aquacultural Engineering, 2nd ed., Wiley-Interscience, New York. 708 pp.

Wimberly, D. M. 1990. *Development and Evaluation of a Low-Density Media Biofiltration Unit for Use in Recirculating Fish Culture Systems*, Master's Thesis, Louisiana State University, Baton Rouge, Louisiana.

Yurkowski, M., and J. L. Tabachek. 1974. Identification, Analysis, and Removal of Geosmin from Muddy-Flavored Trout, J. Fish. Res. Board Can., No. 31, pp. 1851-1858.

Winfree, R. A. 1989. Tropical Fish, World Aquaculture, Volume 20(3), September 1989.

Zajicek, P. 1993. Limited Markets trap The Alligator Industry. Aquaculture Vol. 19, No. 6 pages 50-59.

Zhang, T. C., Y. C. Fu, & P. L. Bishop 1995. Competition for Substrate and Space in Biofilms. Water Environmental Resources. 67:992-1003.

(6) Related Research or Research and Development

AST and/or its principals have been actively involved in R&D efforts directed towards the development of recirculating technologies with an emphasis on the use of floating bead filters for nitrification and solids capture. Through these efforts as well as regular participation in the World Aquaculture Society, Aquaculture Engineering Society, various state sponsored aquaculture conferences, and business relationships with a wide variety of researchers and commercial fish farmers throughout the country, we are well informed of ongoing technological efforts. AST has also recently joined an informal alliance with two other companies: Applied Aquatics, Inc. on the U.S. east coast, and Aquaneering, Inc. on the west coast, to better serve the aquaculture community. This alliance also enables us to keep abreast of new technological advances in the state of the art concerning recirculating systems technologies.

AST is not aware of any other businesses that are activity pursuing the enhancement of floating bead bioclarifiers through manipulation of the media shape or texture for use in aquaculture production systems. A number of inventors (Dr. W. J. Jewel of Cornell University and John Junius of A-1 Aquaculture, Inc.) have investigated the potential use of dynamic "bead" filters but the primary function of these filters is not clarification (although they do capture some solids) and thus are not believed to conflict with our advocation of bioclarifiers. Fluidized bed work is being pursued in several locations, most notably the Freshwater Institute and Cornell University. The use of "biocarriers" has been advocated for use in fluidized beds for a number of years; again, the focus of these works has been biofiltration, not dual function as advocated here.

A number of major research institutions are actively pursuing the development of recirculating technologies. Well known centers that have sustained research in the area over the years include Cornell University, the University of Maryland, Illinois State University, Virginia Polytechnic Institute, Texas A & M University, University of California at Davis, and North Carolina State University. The Freshwater Institute in West Virginia and The U.S. Fish and Wildlife Dworshak National Fish Hatchery facility have also made notable contributions in recent years. In the private sector, Scientific Hatcheries, Inc. of Huntington Beach, California has been a leader in the demonstration of recirculating technologies for tropical fish production.

AST has a Phase I SBIR project entitled "Development of an Extremely Low Water-Loss Recirculating Floating Bead Filter for Biofiltration and Solids Capture on Recirculating Marine Culture Systems." pending with the United States Department of Commerce through the National Sea Grant College Program's SBIR Program. This proposed project addresses the development of a low waterloss "metal free" bubble-washed filtration platform (see Figure 7) for use in marine recirculating systems. A corrosion free bioclarifier is needed for the marine recirculating aquaculture community.

Additionally, AST has been lending support to ongoing projects at Louisiana State University under the direction of our consultant, Dr. Ronald F. Malone. A number of these efforts are funded by and through the Louisiana Sea Grant College Program. Of particular interest is a thrust to develop recirculation formats for marine fingerling production systems currently being conducted with a number of researchers including Dr. Philip Lee of the University of Texas Marine Biomedical Laboratory, Dr. Bill Neill of Texas A&M University, Dr. J. R. Tomasso of Clemson University, Dr. Granvil Treece of Texas A&M University, Dr. Kelly Rusch of Louisiana

LOUISIANA STATE UNIVERSITY
AND AGRICULTURAL AND MECHANICAL COLLEGE
Department of Civil & Environmental Engineering

February 12, 1997

Douglas G. Drennan, III
Managing Member
Aquatic Systems Technologies, LLC
P.O. Box 15827
New Orleans, LA 70175-0827

Dear Doug:

This letter confirms my willingness and availability to serve as a consultant on your proposal entitled "High Frequency Wash Airlift Bioclarifier Using Modified Floating Media for Recirculating Systems". As we have discussed, our ongoing research continues to indicate that the most cost effective floating bead filters will operate with high frequency washing and modified beads.

This work will have to be conducted in accordance with the University's guidelines for consulting activities.

Sincerely,

Ron Malone

Ronald F. Malone, Ph.D., P.E.
Chevron USA Professor

Baton Rouge • Louisiana • 70803-6405 • 504/388-8442 • FAX 504/388-8652

LOUISIANA STATE UNIVERSITY
AND AGRICULTURAL AND MECHANICAL COLLEGE
Department of Civil & Environmental Engineering

February 12, 1997

Douglas Drennan
Aquaculture Systems Technologies, LLC
108 Industrial Avenue
Jefferson, LA 70121

Dear Douglas:

We will be happy to provide analytical support for your proposed research project "High Frequency Wash Airlift Bioclarifier Using Modified Floating Media for Recirculating Systems". The analysis will be conducted in our Water Quality Laboratory in the Department of Civil and Environmental Engineering here at Louisiana State University. All analyses will be conducted in accordance with Standard Methods (See attached Table). The lab has a full time director and participates in the USEPA Interlaboratory Quality Control Testing Program.

The cost for the analysis for the eight parameters you requested is $ 50.00 per sample or $ 300.00 for each set of influent/effluent analysis on the three filters. This price includes the analyses for the parameters listed below, travel time and expenses required for sample pick-up, and preparation and distribution of laboratory reports. Our lab has an income account for work of this nature and a formal contract will not be required. If I cam be of any further assistance please feel free to contact me.

Sincerely,

Christine Higginbotham

Christine Higginbotham
Research Associate

Water Quality Analyses Procedures	
Parameter	Analysis Procedure
Total Ammonia Nitrogen	Distillation and Nesslerization-colorimetric
Nitrite	Diazotization Method-colorimetric
Oxygen	Winkler with Azide Modification
pH	Orion Model 290A Digital meter
Alkalinity	Sulfuric Acid Titration-potentiometric
Biochemical Oxygen Demand	Winkler with Azide Modification
Total Suspended Solids	Vacuum filtration/drying oven 103 C
Turbidity	Turner Designs Nephelometer

Note: All procedures for Standard Methods (APHA, 1989)

Vernon Rodrigue

Vacherie Machine Works, Inc.
2990 LA Highway 20
Vacherie, LA 70090

February 10, 1997

Douglas Drennan
Aquaculture Systems Technologies, LLC
P.O. Box 15827
New Orleans, LA 70175

Dear Douglas:

This letter confirms my willingness to serve as a consultant on your Phase II USDA SBIR Project entitled " High Frequency Wash Airlift Bioclarifier Using Modified Floating Media for Recric. Systems" and assist with the construction of a bead modification device similar to, but with a larger capacity than the one we constructed for Phase I. As you know, I feel that modified beads, in an airlift configuration is the direction you will have to move to realize an increase in the carrying capacity while decreasing the production cost of your filter designs.

Sincerely,

Vernon L Rodrigue

Vernon Rodrigue
Operations Manager

(G) POTENTIAL POST APPLICATIONS

AST believes that the future for recirculating technologies is bright. Many factors, including issues as diverse as bird predation, diminishing water supply, environmental regulations, cost of coastal lands, concerns about exotic introductions, and quality issues such as off-flavor are driving the aquaculture industry towards increased production, at least in part based on recirculating formats. The key to success will be the availability of reliable and cost effective production technologies. Phase II will continue to address the scientific issues, apply findings to development of new technologies, and evaluate prototype systems that may lead directly to the implementation of a new generation of bead filters or "bioclarifiers" utilizing modified floating media.

Successful completion of this Phase II effort will position AST for immediate commercial production and marketing of these revolutionary filters, based on the prototype development. The Phase II effort will culminate in the development and testing of a commercial scale unit (25 cubic feet of media) capable of supporting approximately 10,000 pounds of fish (50 lbs of feed per day) under typical growout conditions. We anticipate that these new units will be fabricated out of polyethylene plastic by the "rotomold" process, which will significantly reduce the cost per cubic foot of filter media over comparable units constructed from fiberglass.

Furthermore, construction of both larger and smaller commercial models is expected to meet the requirements for an expanded range of applications endorsed by the aquaculture industry. AST currently offers six propeller-washed filter models (Model PBF-3, 5, 10, 25, 50 and 100). Additionally, six bubble-washed filter models (Model's BBF-1/4, ½, 1,2,5, and 10) are also currently available, and two more sizes will be added to our product line within the next several months.

The potential uses of the proposed research by the Federal Government are in connection with the research, mitigation, and demonstration programs carried out by federal fish hatcheries and aquaculture research facilities throughout the United States. Bead filter technologies have been well received by these organizations; to date, AST has sold several standard propeller-washed and bubble-washed units to federal installations maintained by the U.S. Fish and Wildlife Service and the U. S. Bureau of Reclamation.

Aquaculture Systems Technologies, LLC (AST) was founded by Douglas Drennan, in October 1995, through the acquisition of assets owned by Armant Aquaculture, Inc. (AAI). AST acquired from AAI certain assets including, but not limited to, the exclusive manufacturing, marketing and sales rights for "Propeller-Washed Bead Filters" and similar non-exclusive rights for the "Bubble-Washed Bead Filters". Both filter configurations are patented, and AST has an exclusive licensing arrangement with the patent holder. Shortly after completing the acquisition in February 1996, the number of commercially available propeller-washed filter models was doubled from three units (PBF-8, PBF-20 and PBF-30) to six units (PBF-3, PBF-5, PBF-10, PBF-25, PBF-50 and PBF-100). AST currently has five full time employees and one part-time employee.

Floating plastic media have been employed since the mid-1970s in the biofiltration components of high-density systems for raising food, game and ornamental fish. Although successful, the earliest air-washed filters were poorly understood and their use was limited. In the late 1980's, researchers demonstrated that hydraulically washed bead filters could perform both solids removal and biofiltration for high density catfish grow-out systems. These results stimulated additional research on bead filters. Development of mechanically washed units (U.S. patent #5126042) overcame many of the operational difficulties experienced by earlier designs. Shortly thereafter, the bubble washed bead filter (U.S. patent #5232586) was developed and tested for use on small research systems and outdoor ponds.

To date over 400 bead filters have been sold and are being used in over 40 states as well as Puerto Rico, Costa Rica, Panama, Canada, England, Japan, and Israel. Since 1989, bead filters have been applied on systems holding foodfish species such as tilapia, catfish, striped bass, trout, flounder, and red drum, as well as systems for ornamental and tropical fish, alligators, crawfish, crabs, oysters, clams and turtles. Since its inception, AST has sold over 250 filter units.

While AST is aware of several other "bead filters" being developed and tested, none of those have achieved prominence in the recirculating systems market. The AST bead filters, including propeller-washed and bubble-washed models, are the only filters on the market today that combine solids capture and biofiltration in the same unit. Even the bead filters being developed independently by entities not associated with AST cannot and do not claim that their filters perform as dual-role bioclarifiers. Micro screen devices, including rotating drum and disc filters, do compete with bead filters somewhat in certain applications that employ bead filters as solids capture devices only, such as when operated in series with fluidized bed. However, due to the complex mechanical nature of the micro screen filters, as well as their excessive water loss during backwash and the practical limits on their solids capture ability associated with screen sizes and biofouling, even this technology is not considered a significant competitor. Additionally, when bead filters are operated in series with fluidized beds, they act as heterotrophic bacterial sumps that capture and hold the organic waste produced by the fish. This action appears to eliminate competition between the rapidly growing heterotrophic bacteria and the nitrifying bacteria for available surface area, and so improves the nitrification function of the filter.

Additional savings are also possible by optimizing the filter platform. An airlift bead filter can be constructed using a non-pressurized vessel, since it operates at less than 5 psi. This will allow us to use a rotationally molded polyethylene tank hull. Our model PBF-3 unit is currently fabricated by this means, with an estimated saving in the filter hull cost of almost 73% on a unit volume basis, as compared with a fiberglass model in a similar size range. In the past, our plastic hull supplier has informed us that the molding of larger filters from plastic would not be feasible because of pressure constraints. The new media, with its increased porosity and consequent reduction in headloss, will now allow us to construct and operate a commercial scale (25 to 50 cubic foot) plastic filter at less than 10 psi. We estimate that a mechanically washed airlift bead filter utilizing modified media will cost approximately 20% less to construct than a standard filter of the same size.

(H) CURRENT AND PENDING SUPPORT

Aquaculture Systems Technologies currently has a Phase I proposal pending with the United States Department of Commerce, National Oceanic and Atmospheric Administration, Office of Oceanic and Atomospheric Research, Silver Springs, MD, 20910. Date of award notification is June 1997. The solicitation subtopic title is "Aquaculture: Water Reuse and Effluent Treatment Systems". The proposal number is 97-1-282. The title of the proposed project is "Development of an Extremely Low Water-Loss Recirculating Floating Bead Filter for Biofiltration and Solids Capture on Recirculating Marine Culture Systems". Douglas Drennan, Managing Member of Aquaculture Systems Technologies, LLC is the proposed principal investigator.

Appendix A

TILTECH AQUAFARM, INC.

45056 RIVERDALE HEIGHTS RD. • ROBERT, LA 70455 • (504) 345-3440 • fax (504) 345-3440

February 10, 1997

Douglas Drennan
Aquaculture Systems Technologies, LLC
108 Industrial Ave.
Jefferson, LA 70121

Dear Douglas:

We are in the planning stages of a major expansion of our tilapia production facility. Plans include construction of eight (8) additional 40,000 gallon grow-out tanks and a quadrupling of our hatchery/fingerling production facilities. We are planning on using your standard commercial bead filters in all phases of our expansion, and would welcome the opportunity to participate in your Phase II USDA SBIR Project entitled "High Frequency Wash Airlift Bioclarifier Using Modified Floating Media for Recirc Systems".

As you know we feel very strongly that airlift technology is the future in recirculating systems. By utilizing air for water movement, aeration, degasification and foam fractionation, we can eliminate centrifugal pumps and realize a significant decrease in production cost. It is also my understanding from your explanation of this research effort that when commercially available, airlift filters will cost about one-third less than your standard filters since they no longer have to be constructed in fiberglass pressure vessels.

We agree to provide you with the use one of our 40,000 gallon grow-out tanks for commercial testing of the airlift, high frequency washed bioclarifier. We will operate the system as we do any of our grow-out tanks and allow you and your research team full access to the system and our facility for data collection.

Please feel free to contact me if I can be of any further assistance.

Sincerely,

Steve Abernathy
President

Appendix B

INNOVATIVE ENVIRONMENTAL SOLUTIONS, LLC.

P.O. Box 8425
Ft. Wayne, Indiana 46825
(219) 492-2061

IES is committed to providing innovative solutions for the environmental concerns of today's businesses. We work with businesses to develop and implement strategies which help to provide a safer environment for all of us to share. We at IES realize that the only plan which can succeed is one which maximizes the benefits to the consumer and through sustainable design insures that the impact to the environment is kept to a minimum. Our ability to meet the customers needs on the individual level make projects succeed. We have an open and direct communication path to the customer which allows suggestions to become designs, and ideas to become reality.

We feel that our niche in the market-place is based on our view that no job is too small. Too many companies never get the opportunity to truly explore their growth potential through technology because of the greedy desires of some big business. We are willing to make just 25 of something, to see if it can open up new market places for small business. In fact, a development project completed in 1995 for only 20 units, lead to sales in 1996 of 1000 custom controllers and anticipated sales in 1997 of over 2000.

The Aquaculture Systems Technologies (AST) Filter controllers are a joint effort of Innovative Environmental Solutions, LLC.(IES), db Engineering and AST, LLC. They were developed to meet the needs of those in the Aquaculture industry for an automated system to keep their filters clean and functioning properly. Additionally, with the use of a computer not only to operate, but to monitor the function of the critical component parts, we are able to provide to the consumer, an automated system which provides much more variability and control over the filter back wash process. In the design we strove not only to automate those tasks which have only been done manually before, but to also add in safety and convenience features which help to run the system more efficiently.

Ron Suchecki of IES is completing his Masters in Environmental Science at Baylor University with a concentration in water and wastewater treatment. His extensive background includes the design, construction and management of an ANSI accredited testing site for the implementation of the ANSI/NSF Standard 40, 1990 and 1996 for Aerobic Wastewater Treatment Systems. His company jointly designed and supplies to Murphy Cormier, General Contractor, Inc. (MCGC) of Lake Charles, Louisiana a custom controller for the operation and monitoring of the Individual Aerobic Treatment plants that MCGC manufactures for sale in the State of Texas. This is a joint project with

Don Brown of db Engineering. Mr. Brown has extensive experience in the semi-conductor and computer industries. In addition to the work with AST, and MCGC, he has designed lift station controllers, automated test equipment for the semiconductor burn-in industry , motor speed controllers and a variety of other equipment. His 25 years of experience in the electronics industry include everything from teaching on the University level to Management and Director positions in the Engineering Departments of a wide variety of International companies in Dallas/Fort Worth Area. His knowledge of computer design and programming are the key to the success of these projects.

IES an db Engineering have had a great experience working with AST in the past and look forward to our future together. We have worked closely throughout the project to ensure the maximum benefit to all those involved. Jointly, our ability to adapt and respond to new ideas has developed into a project which has far exceeded the expectations of all the parties involved. We look forward to the future and enjoy the challenge of what tomorrow may provide.

Sincerely,

Ronald J. Suchecki, Jr.
General Manager

RHINO ROTO MOLDING

CUSTOM ROTO MOLDINGS
We Do
Mold In Graphics

February 12, 1997

Douglas Drennan
Aquaculture Systems Technologies, LLC
P.O. Box 15827
New Orleans, LA 70175

Dear Douglas:

This letter confirms my willingness to work with you on your Phase II USDA SBIR Project entitled "High Frequency Wash Airlift Bioclarifier Using Modified Floating Media for Recric. Systems" and assist with the construction of a prototype airlift bead filter of approximately twenty-five cubic feet in size. I understand that this filter will be similar to, but larger than the Model PBF-3, we constructed for Phase I.

It was a pleasure working with Aquaculture Systems Technologies on prototyping and production of the PBF-3 filtration system for the Phase I SBIR Project. The level of professionalism and expertise was most appreciated.

We would hope to continue our great relationship on your new project.

Sincerely,

Rick Johanneck
President

RJ/sg

400 West Congress • P.O. Box 850
Maple Lake, Minnesota 55358
Phone (612) 963-3996 • Fax (612) 963-6192